Chapter 4

Tests To Diagnose or Predict Disease

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Chapter 4

Tests To Diagnose or Predict Disease

INTRODUCTION

Medical tests that provide information on the present or future development of disease maybe useful adjuncts to the health insurance underwriting process. In general, two types of tests can be distinguished—diagnostic and predictive tests. Diagnostic tests are used to identify the cause of abnormal physical signs or symptoms. In contrast, predictive tests are generally applied to asymptomatic individuals and are used to provide information regarding the future occurrence of disease.

Diagnostic and predictive tests may be used in a medical screening program to identify latent disease or disease predisposition (i.e., it maybe diagnostic or predictive in intent). In general, a screening program involves administering a screening test to an asymptomatic population to sort out apparently well persons who probably have disease (or who have an increased likelihood to develop a disease) from those who probably do not have disease (or probably will not develop disease) (figure 4-1). More definitive tests are then administered to those identified by the screening test as being at risk. Some screening programs employ "non-medical" tests to detect behaviors associated with disability or disease. Examples include tests for drug or alcohol use and nicotine tests to identify current smokers.

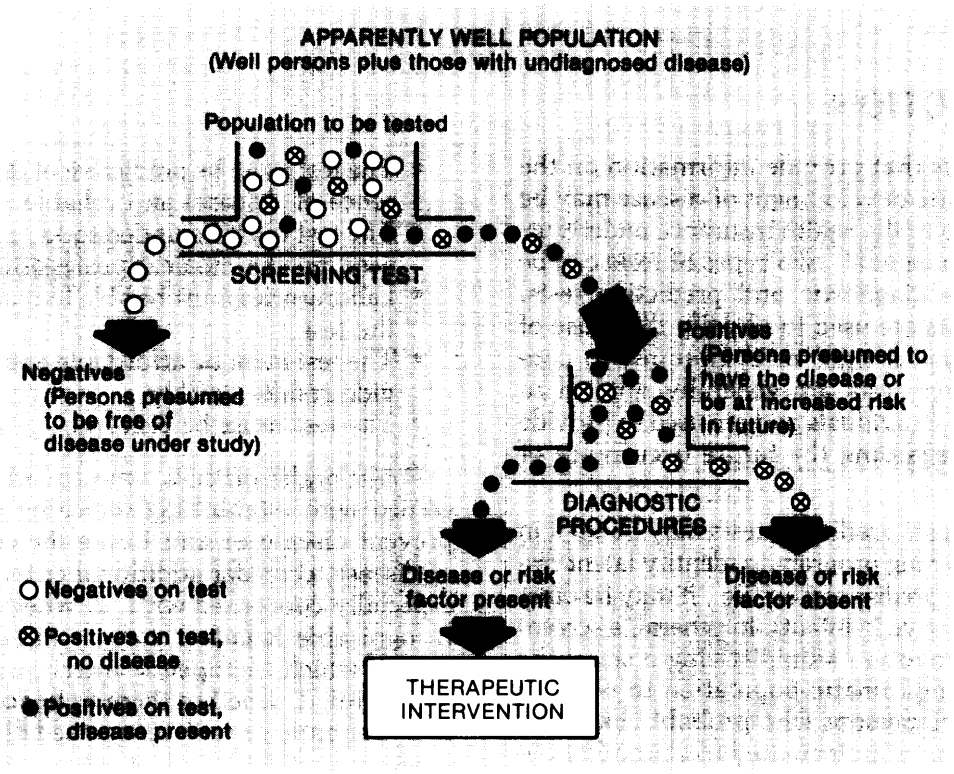
According to one insurance company's position paper on the use of genetic tests and tests for disease predisposition, several conditions should be met before a medical test is adopted by insurers (45).

- The test must supply information in addition to information otherwise available from other sources (e.g., from the medical history questionnaire).
- The disease tested for must have serious morbidity and/or mortality implications.
- The disease must be common enough to ensure that the test is predictive and that the cost can be justified.
- The test must be predictive of disease (or absence of disease) and reliable.
- The test must be understood, accepted, and used by the medical profession.
- Laboratories must be able to readily perform the test.
- The test must be affordable and able to provide results quickly.
- The test must be risk-free.

Public health officials, who are principally concerned with establishing screening programs to prevent disease or ameliorate the consequences of disease, consider additional criteria. They are especially concerned with: 1) whether there is a recognizable latent or early symptomatic stage during which therapeutic interventions may be successful, 2) whether there is an accepted treatment for patients with recognized disease, and 3) whether facilities for diagnosis and treatment are available. Clearly, insurers are mindful of these considerations as well, as they would have little interest in testing for a condition that could be inexpensively and effectively treated. Insurers also consider disease latency; there would be little value in a test that predicted the occurrence of a disease with a late onset (e.g., age 65 or older). Instead, insurers are more interested in tests for diseases that afflict younger persons and that have no effective treatment or are very costly to treat.

For persons applying for individual or small group health coverage, insurers often refuse to insure or offer insurance on a substandard basis to those with evidence of significant disease, including heart disease (e. g., history of angina pectoris, arteriosclerosis) and insulin-dependent diabetes. If predictive tests are developed for diseases that are currently the basis of exclusion or substandard coverage, how likely are they to be used to test healthy applicants? The answer, in part, will depend on the availability of preventive interventions. If interventions are unavailable, predictive tests may only be of interest to insurers if they

Figure 4.1.— Flow Diagram for a Mass Screening Test



SOURCE: J.S. Mausher and A.K. Bahn, *Epidemiology: An Introductory Text* (Philadelphia, PA: W.B. Saunders Co., 1974).

are very accurate. If inaccurate, many applicants not destined to become ill would be excluded or subjected to expensive follow-up testing.

Predictive testing may also be used to establish preventive health plans. For example, the availability of such tests and concerns regarding health cost containment may foster the establishment of employee "wellness" programs. If a predictive test for heart disease is developed and an effective intervention is available, an employee may be tested and encouraged to comply with preventive measures.

At the present time, tests conducted on applicants at the request of commercial health insurers are largely limited to biochemical profiles, tests for Human Immunodeficiency Virus (HIV) infection, and specific drug tests.

TESTS CURRENTLY USED BY HEALTH INSURERS

Most of the blood tests used by health insurers are those biochemical profiles used frequently by clinicians. Such profiles are generally a battery of twelve or more tests that are performed on each blood sample as part of a large-volume, automated-testing program. Although it is recommended that medical tests be administered on the basis of clinical findings, the ease with which a

large number of tests can be conducted at relatively low cost has led in part to the routine use of biochemical profiles. For example, biochemical profiles have been used to screen asymptomatic patients in ambulatory clinics and as part of routine hospital preadmission workups.

Table 1-1 in chapter 1 identifies the blood and urine tests conducted at a major laboratory that serves commercial insurers and the conditions the tests may detect. The sensitivity of these tests, or the ability of the tests to correctly identify those with the corresponding conditions, depends on the particular tests. For example, the total serum protein level is not very sensitive for any of the conditions with which it is associated. In contrast, increased levels of high glucose (sugar) are almost always detected in those with diabetes mellitus.

An abnormal result is usually defined by setting a cutoff value, beyond which values are deemed abnormally high (or low). In general, a result that deviates markedly from the mean value for a given population is more predictive of disease than one that deviates only slightly. However, when the cutoff value is established by using certain statistical criteria (such as the mean value for the test in a population of presumably nondiseased persons, plus or minus two standard deviations), as the number of tests administered increases, the likelihood that an individual will, by chance, have at least one abnormal test result also increases (table 4-1).

If the test is positive, how likely is it that the person tested has the condition in question; and if the test is negative, how likely is it that the person is disease-free? Stated another way, what is the test's predictive value, or the probability that a positive test correctly identifies the presence or the future development of the indicated condition or disease?

Table 4-1.—The Probability That a Healthy Person Will Have at Least One Abnormal Result in a Biochemical Profile

Number of tests	Probability that at least one test will be abnormal (percent)
	5"!0
:	26
12	46
20	64
100	99

SOURCE: Adapted from RD. Cebul, and JR. Beck, "Biochemical Profiles: Applications in Ambulatory Screening and Preadmission Testing of Adults, " Common *Diagnostic Tests: Use and Interpretation,* H.C. Sex, Jr. (cd.) (Philadelphia, PA: American College of Physicians, 1987).

In one study of 8,651 patients who were tested **as** part of a multiphasic health checkup, use of a biochemical profile consisting of 8 tests resulted in 26 percent of patients having at least 1 test abnormality. In the case of serum glucose, although 6 percent of adults had elevated levels, less than 30 percent of them had elevations when the test was repeated. Furthermore, disease was confirmed in less than 17 percent of those with repeatedly abnormal test results (96). Similarly, approximately 3 to 4 percent of asymptomatic adults will have abnormal serum creatinine and blood urea nitrogen levels, but few will have actual kidney disease (44).

The predictive values of biochemical profile test results among patients about to be admitted to the hospital are comparable to those cited above. Experience from such programs indicates that 40 percent of such patients will have abnormal tests but that these results will lead to new diagnoses in only approximately 4 to 10 percent of patients (44).

The Blue Cross and Blue Shield Association has published guidelines on the use of biochemical profiles in both ambulatory settings and hospital preadmission testing programs (these guidelines were written with the cooperation of the American College of Physicians, the American College of Radiology, and the American College of Surgeons). The guidelines state that biochemical profiles are not routinely indicated for screening asymptomatic adults or those without risk factors, nor are they indicated prior to elective admission to the hospital (207). However, the guidelines state that selected components of biochemical profiles may be indicated for screening asymptomatic adults; specifically, serum glucose (to identify diabetes mellitus), serum cholesterol (to ***iden***tify hypercholesterolemia), and serum creatinine, with or without blood urea nitrogen (BUN) (to identify kidney dysfunction).

Insurers usually selectively test those with medical histories indicative of disease or risk of disease. If a relatively high-risk group of individuals is tested, the predictive value of the tests would be expected to be higher than in unselected testing. There is evidence that insurance applicants are being selectively tested. For example, according to 1986 insurance testing data from the Home Office Reference Laboratory, Inc. (HORL) which conducts tests for more than 80 percent of life and health insurance companies in the United States and Canada, 15 percent of applicants between the ages of 20 to 59 who were tested had abnormal blood glucose levels (246). In contrast, in a study performed by others of unselected adult patients tested as part of a multiphasic health checkup, only 6 percent had abnormal blood glucose levels (96).

While controversy surrounds the use of tests for HIV infection by insurers, insurers are selectively testing life and health insurance applicants when permitted to do so. HORL, the lab that does most of the testing for the insurance industry, uses state-of-the-art technology in the operation of a high-volume, largely automated laboratory. When applicant blood specimens are sent from locales in which HIV antibody testing is permitted, HORL uses the recommended two-stage testing protocol. First, an enzyme-linked immunosorbent assay (ELISA) is used to test serum or plasma for the presence of antibodies to HIV. These tests are very sensitive and therefore detect nearly all of those who have produced antibodies to HIV. However, they falsely identify as positive some that have not been infected. To more accurately identify noninfected individuals, a confirmatory test (sometimes called a supplemental test), the Western blot, is used. In 1986, 128,129 HIV antibody tests were performed by HORL (for a total of 213,193 life or health insurance applicants). Of those tested, 385 (0.3 percent) were Western blot-confirmed as positive. Among the 13,7s9 applicants in the 20-to-29 year-old group, 8,312 were tested for the presence of antibodies to HIV and of these, 85 (1 percent) were confirmed as HIV infected (246). Table 4-2 summarizes HORL testing for HIV antibodies by age groups for 1986.

When HIV antibody testing is prohibited (e.g., in California), insurers test applicants by using a surrogate test, the T-lymphocyte (or T-cell helper-suppressor ratio) test. This test of immune function is much less accurate than the antibody test in identifying HIV-infected individuals. According to a study conducted by HORL, the T-cell test failed to detect 18 percent of 234 specimens that had tested positive for HIV antibodies (113). (Furthermore, a study of 65 asymptomatic HIV antibody-positive blood donors done elsewhere revealed that none had abnormal T4/T8 cell ratios (161).) To determine the predictive value of the T4/T8 ratio, HORL tested 209 specimens with T4/T8 ratios less than 1.0 for HIV antibodies. Only 8 percent of specimens were confirmed positive for antibodies. The predictive value increased somewhat as the T4/T8 ratio decreased (113).

The T-cell test may be abnormal when there is no HIV infection, because it is a general test of immune function. HORL'S 1986 testing data indicate that 1 percent of the 25,611 T-cell tests conducted (for a total of 213,193 life and health insurance applicants) were positive. Table 4-3 summarizes HORL T-cell testing by age groups for 1986. Note the relatively high percent of positive tests in the older age groups, who would be least expected to be HIV antibody-positive.

Table 4-2.—Age-Specific HORL HIV Antibody Test Statistics for 1986

Age group	Number of tests	Number of positive tests	Percent of positive tests
1-19	395	1	0.250/o
20-29	8,312	85	1.02
30-39	35,417	173	0.49
40-49	38,831	75	0.19
50-59	31,226	46	0.15
60-up	13,948	5	0.04
Total	128,129	385	0.30 "/0

SOURCE: Home Office Reference Laboratory, Inc. (HORL), unpublished data, Shawnee Mission, KS, 1986.

Table 4-3.—Age-Specific HORL T.cell Test Statistics for 1986

Age group	Number of tests	Number of positive tests	Percent of positive tests
1-19	48	0	0.00"!0
20-29	1,379	24	1.74
30-39	6,377	86	1.35
40-49	8,504	76	0.89
50-59	6,291		1.17
60-up	3,012	; ;	1.00
Total	25,611	290	1 .13 "/0

SOURCE: Home Office Reference Laboratory, Inc. (HORL), unpublished data, Shawnee Mission, KS, 1986.

While some insurers are prohibited from performing HIV antibody tests they are not explicitly prohibited from using HIV antigen tests. Several commercial products are available for research use (at present, none are FDA-approved for diagnostic purposes). Such tests have been useful in detecting early infections at a time before HIV antibodies are detectable. However, once antibodies are produced, the antigen test may be negative. Therefore, such a test would not be as useful as the antibody tests, and there is no evidence that insurers are using such tests.

TESTS FOR HIV

The ELISA and Competitive EIA Screening Tests

Several manufacturers have been licensed by FDA to market HIV screening test kits. Most of the test kits are enzyme immunoassay (EIA) that identify IgG antibodies (one of several classes of antibodies) made in response to HIV infection. The EIA tests (ELISA tests area type of EIA) were initially designed to screen blood products, and according to data submitted to FDA by the manufacturers, most kits can detect virtually all individuals with AIDS (table 4-4). However, the tests will not identify recently infected individuals who have not yet produced antibodies to the virus. Furthermore, during the early stages of infection, some individuals (e.g., newborns) make antibodies (IgM) that are not detected by the available tests. However, almost all infected individuals will test positive 1 to 4 months after infection (60).

Table 4-4.–Commercially Available (U.S.) HIV Screening Test Kits[a]

Manufacturer and test name	Sensitivity[b]	Specificity[c]
Abbott Laboratories		
Abbott HTLV III EIA	100%	99.85%
Cellular Products, Inc.		
Retro-Tek™ HIV ELISA	100	99.85
El. du Pent de Nemours & Co., Inc.		
Du Pent HTLV-111 ELISA	99.30	99.70
Electro-Nucleonics, Inc.		
VIRGO™ HTLV-111 ELISA	100	99.68
Genetic Systems		
Genetic System LAV EIA™	100	99.80
Organon Teknika, Corp.		
Bio-EnzaBead ™ HTLV-111 ELISA	100	99.97
Ortho Diagnostics Systems, Inc.		
ORTHO™ HTLV-111 ELISA	99.30	99.70

aAvallable ~~ of early 1988. Several other HIV screening test kits will be available pending FDA approval (e.g., Hoffman. La Roche, Cambridge Bioscience)
bSensitivity is computed as percentage of AIDS patients testing positive assuming 100°/0 prevalence of HIV antibody in AIDS patients
cSpecificity is computed as percentage of random blood donors testing negative assuming zero prevalence of HIV antibody in random blood and plasma donors.

SOURCE Office of Technology Assessment, 1988

False-positive tests may occur, because the tests use disrupted whole-virus preparations derived from cell cultures as the antigen (viral components capable of eliciting an antibody response). Although made from purified virus, they are contaminated with cellular matter that can produce false-positive results. The cutoff levels above which a test will be interpreted as positive are set low in order to detect as many positive specimens as possible, but this also increases the chances that a specimen labeled as positive at these low levels might in fact be negative. Therefore, a specimen is not reported as positive until appropriate follow-up tests are conducted. This includes a repeat of the initially positive screening test. In fact, the repeated ELISA is done in duplicate. Only if one of the two repeat tests is positive will the serum specimen be reported as ELISA positive (or repeatedly reactive), and a confirmatory test subsequently performed. The problem of false positives attributable to contamination will be reduced when "second generation" test kits become commercially available. Instead of disrupted whole virus, these kits will use synthetic viral antigens made by using recombinant DNA techniques, thereby avoiding the problem of contamination.

Confirmatory Tests for HIV Antibodies: The Western Blot

To confirm a positive ELISA screening test, a technique called the Western blot is used. Here, purified HIV antigens are separated electrophoretically on a gel and then blotted onto special paper. A sample of blood is applied to the paper, and, if antibodies are present, they will bind to the viral antigens and appear as distinctive bands

on the blot. The location of each band indicates reaction with a specific viral protein. There are three types of HIV proteins: 1) proteins that provide the virus's internal, or "core" structure; 2) proteins that provide the external or "envelope" structure; and 3) proteins that are the enzymes (for example, reverse transcriptase) that the AIDS virus uses to regulate interactions with its host cell. The core proteins include p24/25 and p55, which are shorthand designations for proteins with molecular weights in the thousands (or "kilodaltons"). Thus, p24/25 refers to a protein with a molecular weight of 24 or 25 thousand. The envelope proteins are gp41, gpl10/120, and gp160, where "gp" stands for "glycoprotein" (envelope proteins have non-protein elements—glycogen—incorporated in them), and p17/18 (previously thought to be a core protein). The regulatory enzyme proteins are p31/32, p51/53, and p65/66.

Early in the AIDS epidemic a Western blot was interpreted as positive even when antibodies to only one of the proteins (the core protein, p24/25) of the AIDS virus was present, but it soon became evident that the blood of noninfected persons could contain similar antibodies. Reactivity to core antigens exclusively may represent infection with another retrovirus (e.g., HTLV-1, which may cause a particular type of leukemia) or reactions with other substances. In such cases, a second confirmatory test is conducted in 4 to 6 months, by which time the subject should have produced antibodies to other antigens if HIV infection is indeed present.

The Western blot is visually interpreted, and a weakly reactive band may be read as positive or negative, depending on the technician. Most laboratories limit positive readings to those blots that have reactions with at least two bands, at least one of which must bean envelope antigen. A 1986 National Institutes of Health Consensus Development Conference concluded that the presence of antibodies to two HIV proteins, p24/25 (a core protein) plus gp41 (an envelope protein), constituted an "unequivocally positive" Western blot (30s). However, this conclusion is under dispute, and different laboratories currently have different standards. For example, at the beginning of 1988, the American Red Cross required antibodies to at least one protein from each of the three types to be present before donors are notified that they have tested positive (85). (The Red Cross nevertheless discards all repeatedly positive ELISA blood donations.) The only commercially licensed Western blot test as of early 1988 (Biotech/duPont HIV Western Blot, Biotech Research Laboratories, Inc.) is interpreted as positive when antibodies to p24/25 (a core protein), p31/32 (a regulatory enzyme protein), and either gp41 or gp120 (both envelope proteins) are present (72). The Department of Defense (35) has adopted the definition established by the Association of State and Territorial Health Officers (ASTHO) (positive if any two of p24/25, gp41, or gpl10/120-gp160 bands are present) (104). Given the subjective nature of Western blot interpretation and variations in the definition of a positive result, the establishment of a national standard for Western blot interpretation has been recommended (196).

Other Confirmatory Tests for HIV Antibodies

When Western blot confirmatory tests are equivocal, the radioimmunoprecipitation assay (RIPA), a research procedure, maybe used. However, it is expensive, uses radioisotopes, and requires considerable technical expertise. One State Health Department (California) is using the indirect immunofluorescence assay (IFA) as a confirmatory test at HIV counseling and testing sites. This test relies on the reaction of serum HIV antibodies to HIV virus present in laboratory cultured HIV-infected cells. When serum is added to the infected cells, any antibody that is present binds to the viral antigens. Fluorescein-labeled goat antihuman globulin is then used to detect the presence of intracellular HIV antibodies. Results are read with a fluorescent microscope (253). A commercial indirect immunofluorescence assay was being evaluated by the Food and Drug Administration (FDA) as a confirmatory test as of early 1988 (IND application submitted to the FDA) (184).

An alternative to the usual Western blot confirmatory test is now available, using six recombinant DNA-derived HIV antigens (3 are proteins derived from gp120 and gp41; 2 are portions of core proteins p24 and p55; and one is a peptide derived from polymerase proteins). The test, called

Hivagen, is available as a laboratory service through SmithKline Beckman. Advantages over the usual Western blot include reduced false positive and indeterminant results and an ELISA format, which allows for automated testing and objective interpretation of results (242). Reactivity to envelope antigen plus either core or polymerase (enzyme) antigen constitutes a Hivagen-positive test (190).

Tests To Detect the Presence of HIV

There are methods available to detect the presence of HIV itself instead of antibodies to HIV. Direct observation of the virus or signs of viral activity can be made following successful culturing of HIV. However, culturing peripheral blood mononuclear cells may take 2 weeks or longer and is expensive and technically difficult to perform. However, one company has established a commercial laboratory dedicated solely to AIDS testing and eventually plans to use a semi-automated culturing technique. At present the laboratory uses a manual process that provides results in 6 to 14 days (188).

h ***situ*** hybridization involves the use of radioactively labeled probes to identify HIV-produced RNA or DNA. These genetic probes, produced through recombinant DNA technology, are complementary to the virally produced genetic material and therefore align to and hybridize with it. The method was previously of limited utility, because very few circulating white blood cells are infected with HIV. However, Cetus Corporation has recently developed a method for greatly amplifying the number of infected cells from a few infected cells (82). In ***situ*** hybridization has been used to diagnose HIV infection in newborns. (As mentioned previously, difficulties arise in diagnosing infants of HIV-infected mothers, because the mother's antibodies to the virus are transferred to the infant during pregnancy. Assays for IgM antibodies, which might be used to differentiate the infant's antibodies from its mothers (i. e., IgG antibodies) are also under development to assist in the diagnosis of HIV infection in the newborn.

Indirect Methods To Test for Possible HIV Infection

Before HIV antibody tests were available, some blood banks screened donors using a test for antibodies against the hepatitis B core antigen following reports that as many as 80 percent of AIDS patients had evidence of previous hepatitis infection. At least one blood bank determined T4/T8 lymphocyte ratios to identify possibly immunosuppressed donors. As previously described, some insurance applicants are being tested for T-cell abnormalities. T-cells have characteristic surface markers (antigens). T4 cells (helper-cells) carry the CD4 antigens, and T8 cells (suppressor-cells) carry CD8 antigens. The AIDS virus has an affinity for the CD4 antigen on T lymphocytes and consequently, individuals with various manifestations of HIV infection often have a deficiency of T4 cells and a reversal of the usual ratio of T4 to T8 cells. The T4/T8 ratio can be measured by an automated method of sorting and counting labeled T-cells (flow cytometry, using a fluorescence-activated cell sorter).

The T4/T8 cell testis not very predictive of HIV infection. Advanced age and acute infections are associated with positive test results. And as described earlier, the T-cell test does not accurately identify those that are infected.

There are two other indirect tests of Human Immunodeficiency Virus (HIV) infection. The measurement of urinary or serum neopterin and betaz-microglobulin levels has been described as immunological tests that may be useful in the diagnosis of viral infections, including HIV. Both neopterin and betaz-microglobulin are markers for activation of cell-mediated immunity. While elevated neopterin and $beta_2$-microglobulin levels have been noted in individuals with AIDS and American Red Cross (ARC), elevated levels have not been consistently associated with the HIV-infected, but asymptomatic, state (129). tiurthermore, both markers are non-specific. For example, neopterin is elevated in many individuals with bacterial and viral infections (e.g., staphylococcal pneumonia) (222). The medical community

rarely uses these tests for diagnostic purposes, and there is no evidence that insurers are using neopterin or betaz-microglobulin levels in underwriting applicants.

Accuracy and Reliability of Commercially Available HIV Screening Tests

HIV testing errors may occur because of intrinsic limitations of the tests themselves, laboratory errors in performing the tests, mislabeling, and inaccurate communication of results. The accuracy of a diagnostic test is usually measured in terms of its sensitivity and specificity. Sensitivity is a function of how well a test correctly identifies affected individuals, and specificity describes a test's ability to correctly identify those that are unaffected (figure 4-2). A sensitivity of 99.3 percent means that for every 1,000 screening tests on positive specimens, on average 7 would be incorrectly identified as negative. A specificity of 99.7 percent means that for every 1,000 negative samples screened, on average 3 would be incorrectly identified as positive.

There is no "gold standard" against which the performance of new screening tests for HIV infection can be compared. Instead, measurements of test sensitivity and specificity are based on testing those with clinically diagnosed AIDS and those without known exposures or risk factors. Using these populations, the ELISA HIV-antibody tests are between 99 and 100 percent sensitive and specific (see table 4-4). However, these measurements would be flawed if some "normal" specimens assumed to contain no HIV antibodies indeed contained them. Moreover, some persons meeting the clinical definition of AIDS do not have detectable levels of HIV antibodies.

A number of investigators have evaluated the performance of ELISA screening tests by applying the tests to Western blot-confirmed positive and negative samples, rather than reporting the performance of tests when applied to presumptively positive and negative samples. When evaluated against Western blot-confirmed samples, the sensitivity of commercially available tests ranged from 97 to 100 percent, and the specificity, from 70 to 100 percent (117). Differences have also been noted between the ability of various commercial test kits to identify early infections (254). Some investigators have reported variations in test results when identical ELISA kits from the same manufacturer have been used by different laboratories; and within a lab, batch-to-batch variation has occurred (224).

The predictive value (the percent of positives that are true positives) improves with the prevalence of infection among those screened. For example, suppose the ELISA test can be conducted with a sensitivity of 100 percent and a specificity of 99.8 percent. If the prevalence of antibodies against HIV in the tested population was 0.1 percent (1 in 1,000), only one-third of positive ELISA tests would actually be positive. In contrast, if the prevalence were 10 percent, 98 percent of positive ELISA tests would be truly positive (see fig-

Figure 4-2.—Results of Screening Test Illustrating Sensitivity and Specificity

Result of screening Test	Disease state: Disease	Disease state: No disease
Positive	*true positive* TP	false positive FP
Negative	false negative FN	*true negative* TN

$$\text{Sensitivity} = \frac{TP}{TP + FN} \qquad \text{Specificity} = \frac{TN}{TN + FP}$$

Percentage sensitivity = **percentage of people with the disease who are detected by the test** $= \frac{TP}{TP + FN} \times 100$

Percentage false negatives = **percentage of people with the disease who were not detected by the test** $= \frac{FN}{TP + FN} \times 100$

Percentage specificity = **percentage of people without the disease who were correctly labelled by the test as not diseased** $= \frac{TN}{TN + FP} \times 100$

Percentage false positives = **percentage of people without the disease who were incorrectly labelled by the test as having disease** $= \frac{FP}{TN + FP} \times 100$

NOTE: Predictive value of a positive test $= \frac{TP}{TP} \times 100$

SOURCE: Office of Technology Assessment adapted from J. Mausner and A. Bahn, *Ep'dernio/ogy: An Introductory Text,* 2nd ed. (Philadelphia, PA: W.B. Saunders Company, 19S5).

ure 4-3 and table 4-5). Therefore, even with a highly sensitive and specific screening test, errors will occur, and errors will increase as populations with lower and lower levels of infection are screened (196). Confirmatory tests are therefore necessary to avoid falsely identifying persons as being infected.

The Western blot is much more specific than the ELISA and is therefore useful in correctly identifying those that are truly negative. However, both false positive and false negative Western blots have been reported. For example, as a part of the U.S. Army quality assurance program for HIV testing, a panel of fifteen repeatedly negative serums from healthy adults were sent to five commercial laboratories offering HIV Western blot testing. Six different specimens were classified as positive (four of five of the labs made at least one error; one lab made three errors). This suggests that the errors were due to technique and not to intrinsic biologic properties of the specimens. In addition, five confirmed positive samples were sent to each of the five laboratories. One laboratory falsely identified an HIV positive specimen as negative. In light of these findings, the U.S. Army has adopted a number of policies aimed at minimizing errors in the interpretation of Western blot tests (36).

Figure 4.3.— Predictive Value Calculation for Prevalence of 10 Percent; Test Sensitivity = 100°/0, Specificity = 99.8°/oa

	Antibody present	
	Yes	*No*
Test positive	True positive	False positive
No	0	8,982
	1,000	9,000

Predictive value = @ = 98.2 percent
1,018

aAssume that 10,000 persons are tested.

SOURCE: Office of Technology Assessment, *1988*

Table 4-5.—Relationship Between Predictive Value and Prevalence of the Index Condition in the Population Being Screened[a]

Prevalence	Predictive value of a positive test
10 percent	98.2 percent
5 percent	98.3 percent
1 percent	83.5 percent
0.1 percent	33.2 percent
0.01 Dercent	4.8 percent

aAssumes test sensitivity of 100 percent and specificity of 998 Percent

SOURCE Off Ice of Technology Assessment, 1988

According to proficiency testing data, the performance of HIV testing is not as accurate under "usual" conditions of use as that reported under ideal conditions of use. Results of the College of American Pathologists' (CAP) proficiency testing program from more than 500 laboratories participating in the 1986 and 1987 CAP surveys reveal that of 6,946 ELISA HIV-antibody tests on reactive samples, 99.5 percent were reported as positive and on the 1,142 HIV-antibody negative samples, 98.3 percent were interpreted as negative.

For the Western blot test, the results of only the October 1987 test were analyzed, consisting of three reactive and one nonreactive samples. Of the tests on the 3 reactive samples, 89.2 percent (215 of 241 tests) were interpreted correctly as positive; 23 were reported as indeterminant and 3 were reported as negative. Of the 58 tests performed on the nonreactive sample, 94.8 percent (55 of 58) were correctly interpreted as negative; 3 were reported as indeterminant. The performance of reference laboratories (selected laboratories with good performance records) was more accurate for ELISA and much more accurate for the Western blot tests than was the performance of the other participating laboratories. None of the laboratories participating in Western blot testing reported a negative specimen as positive. When they erred, the results were reported as indeterminate. However, as only one nonreactive sample's results were analyzed, whether labs have in fact never reported nonreactive samples as positive by Western blot is not known. Unfortunately, performance with the licensed versus unlicensed Western blot tests could not be compared, because the data were not collected (206).

These results most likely underestimate problems in HIV-antibody testing, as the proficiency

testing was "open' '-i.e., the laboratories knew they were being evaluated and knew that these were test samples. "Blind" performance testing in which participating laboratories are unaware that they are being evaluated would be a more accurate assessment of laboratory HIV-antibody testing proficiency. (In a further effort to assess the quality of the performance of HIV tests, the Center for Disease Control (CDC) will also implement a nationwide performance evaluation program for HIV antibody testing, but this program too will be of the "open" type, and participation in the program will be voluntary (205).

Advances in HIV Screening Technology

A number of new screening products are under development that will improve the accuracy of HIV testing, and some of these tests are already available for research use.

"Second generation" antibody screening test kits that contain viral components derived from genetic engineering techniques are likely to reduce the number of false positive screening results attributed to contaminants from cell culture present in the first generation tests. Some evidence suggests that these assays may detect infection earlier than the first generation tests (166). In addition to being possibly more accurate than the first generation tests, some of the second generation kits will take less time to process (5 to 30 minutes as opposed to 2 to 4 hours) and may be less expensive.

These very specific HIV-antibody tests may eventually replace the Western blot confirmatory test. An ELISA that uses a short, synthetic peptide that mimics the immunoreactivity of whole HIV is currently being investigated. Nonspecific reactions leading to false positives on Western blot would be eliminated, because a single HIV immune site would be used. However, false-negatives may occur if there are variant HIV strains without the specific immune site. To overcome this problem, a panel of synthetic peptides may be used that covers all HIV strains (192).

Although not yet commercially available, two companies have developed antigen enzyme immunoassay to detect p24 antigen. The presence of endogenous antibodies to HIV interferes with such assays and therefore limits their use (i.e., after an individual produces HIV antibodies, the antigen test may be negative). The HIV antigen enzyme immunoassay has been used to diagnose acute HIV illness in high-risk patients at a time when they have not yet developed HIV antibodies (155). Although the sensitivity and specificity of the HIV-antigen immunoassay are as yet unknown, they may be useful in screening blood products, identifying acute HIV infections, and monitoring the course of therapy for AIDS/ARC patients.

Genetic probes are being developed to recognize viral DNA or RNA sequences (viral genetic material) in cells (209). (Genetic probes are labeled gene sequences synthesized to be complementary to viral sequences.) Probe-based tests will be useful in identifying HIV-infected individuals who do not have detectable virus in their blood. While there are technical difficulties that remain in perfecting DNA probe tests, some of these difficulties have been surmounted. As mentioned previously, the Cetus Corporation has developed a process whereby viral DNA sequences can be multiplied a million times, making it possible to detect viral genes even if present in only one of every 5,000 cells (82).

All of the commercially available screening tests are performed on blood or serum samples. One team of investigators has applied a variant of these tests (IgG-capture radioimmunoassays and ELISA assays) to saliva samples. Almost total qualitative agreement was found in results between paired serum and saliva samples (pairs of samples were both identified as positive or negative); but for quantitative agreement, actual test values for paired samples were not highly correlated (229). Finally, recent investigations have determined that HIV antibodies can be found in urine, and whether this finding will be useful in using urine for HIV-antibody testing is under investigation (40).

Table 4-6 identifies the HIV diagnostic products under development as of early 1988.

Table 4-6.–HIV Diagnostic Products Under Development in Spring 1988

Manufacturer	Product name	Indication
Products pending FDA approval:		
Abbott Laboratories	to be announced	detects HIV antigens
Abbott Laboratories	Envacor	detects antibodies to core antigen p24 and the envelope antigen p41
American Bionetics	Wesblot	automated Western blot
Cambridge Bioscience (Worcester, MA)	Recombigen Latex HIV (rapid HIV antibody test)	detects HIV antibodies
Cambridge Bioscience	Recombigen EIA HIV (two-hour immunoassay)	detect HIV antibodies
Du Pent	HIV p24 core antigen test	detects HIV p24 core antigen
Elect ro-nucleonics	Virgo HIV IFA (immunofluorescence assay)	detects HIV antibodies
Hoffman-La Roche (Nutley, NJ)	to be announced	detects HIV antibodies
Products in development:		
Cetus (Emeryville, CA), Eastman Kodak	SureCell	detects HIV antibodies
Cetus, Eastman Kodak	to be announced	amplifies and detects HIV viral **DNA**
Syntex/Syva (Palo Alto, CA), Cambridge Bioscience	to be announced	test for **AIDS** antibodies
Viral Technologies (Interleukin-2, Alpha-1 Biomedical) (Washington, DC)	to be announced	detects HIV p17 antibodies
Products in clinical trials:		
Chiron (Emeryville, CA), (Ortho Diagnostics, marketer)	RIBA HIV216	validates results of positive ELISA test
Du font	Rapid HIV antibody test	detects HIV antibodies
MicroGeneSys (West Haven, CT)	MGSearch HIV-160	detects HIV antibodies
Thermascan (New York, NY)	Fluorognost (immunofluorescence assay)	HIV-1 antibody confirmation test
Products in research:		
Gen-Probe (San Diego, CA)	to be announced	test for **AIDS** virus
SyntexlSyva	to be announced	test for AIDS virus

SOURCE: Adapted from Pharmaceutical Manufacturers Association, "Pharmaceutical Industw Response to the AIDS Cnsls, " Washington, D. C., Feb. 19, 1988

HIV Self-Tests

Tests for HIV infection that can be performed at home are not currently available, although tests have been developed that could be used as home tests. For example, Cambridge Bioscience has developed a rapid test for HIV infection that can be used on whole blood. The company plans to limit sales to physician offices and health clinics (219). Although tests have been developed that may be simple enough to use on samples collected at home, they have not been approved by FDA fer these purposes. In fact, the FDA has notified companies planning to enter the self-testing market of medical guidelines restricting HIV test kit

use to professionals working within comprehensive health care environments (107).

Several companies had planned to sell kits that allowed the purchaser to collect his own blood, send it anonymously in a prepaid package to a clinical laboratory, and obtain the results of ELISA screening tests (without Western blot confirmation) by phone (189). People anxious to learn about their antibody status but reluctant to see their physicians or to use alternate test sites were expected to use such services (193). Among the concerns that FDA raised in considering mail-in tests were: 1) test results would be provided with limited or no counseling, 2) confirmatory testing was not being offered, 3) the quality of testing would depend on the integrity of mailed samples, and 4) as health professionals would not be involved in the testing process and testing would be anonymous, compliance could decline with the requirement to report the names of those testing positive to State Health Departments in States that have this requirement (e.g., Colorado and Arizona).

GENETIC TESTS

Introduction

Advances in molecular genetics have led to the development of a number of new diagnostic and therapeutic products. Human insulin, growth hormone, and promising drugs for individuals with heart disease have been developed through recombinant DNA technology. In the area of diagnostics, this technology has been used to improve a number of tests for infectious diseases, including HIV tests. While several recombinant DNA diagnostic tests are now being marketed for infectious disease applications, a larger market for diagnostics may be realized when tests for common disorders with a genetic component are developed. Several tests for relatively rare genetic conditions are already available using this new technology. However, they rely on relatively sophisticated techniques, are difficult to interpret, and are therefore available at only a few specialized laboratories. As technological hurdles are overcome, and as advances in molecular genetics continue to be made, new genetic tests may revolutionize the practice of medicine. Tests will improve the diagnosis of suspected genetic disorders and may be widely applied to identify those predisposed to common disorders with a genetic basis. In some cases, early intervention will prevent or ameliorate manifestations of the diagnosed condition.

Although many genetic disorders are rare, collectively they constitute a major source of morbidity and mortality. Evidence suggests that specific genes predispose individuals to some forms of diabetes, heart disease, cancer, and mental illness (267). When the prevalence of these conditions is considered, the potential impact of genetic tests becomes clear. Table 4-7 summarizes one market prediction of the number of DNA-probe tests by type of disease that maybe in use by 1992. Table 4-8 summarizes the projections of several recent market forecasts. These projections probably overestimate the 1992 genetic test market (42) but indicate that tests may soon be available for a variety of genetic disorders and predispositions, and when available, increasingly used in medical practice. As some of these tests are for can-

Table 4-7.—DNA Probe Test for Inherited Diseases

Disease	Number of tests per year	Value (millions)
Purdy gmtic disoasos:		
Adult polycystic kidney	250,000	$ 7.5
Cystic fibrosis	333,000	10.0
Duchenne muscular dystrophy ,.. .,	333,000	10.0
Familial hypercholesterolemia . .	250,000	7.5
Familial polyposis	165,000	5.0
Huntington's disease. ., ., ., ...	20,000	0.6
Neurofibromatosis	250,000	7.5
Retinoblastoma, ., ..., .,	250,000	7.5
Sickle cell anemia ., ., .,	250,000	7,5
Other . . . . . ., .,	500,000	15.0
Subtotal . . . . . ,.,	2,601,000	$78.1
Common disoasas with a gonotic componont:		
Alzheimer's disease.	1,000,000	$30.0
Cancer . . . . . .,	12,000,000	360.0
Diabetes mellitus ., ., . . .,	5,000,000	150.0
Heart disease, ., ., . .	12,000,000	360.0
Subtotal . . ,, ... , ...	30,000,000	$900.0
Totai ..,. .., ., ., ~9OO.O	32,601,000	$950-1,000

SOURCE: Genetic Technology News, "Market for DNA Probe Tests for Genetic Diseases," Gen. Tech. News, pp. 6-7, November 1966.

Table 4-8.—Genetic Test Market Projections

Type of disease[a]	U.S. market value ($ million)	By projected year	Source
Genetic & genetic predispositions	210	—	Biomedical Business International, 1986
Genetic	500	1993	Genetic Engineering News, 1986
Genetic (includes laboratory revenues and identity testing)	550	1990	Robert S. First, 1986
Genetic, infectious and cancer	300-500	1995	Genetic Engineering News, 1987
Genetic	150	1995	Frost&Sullivan, 1985
Genetic &genetic predispositions	950-1,000	1992	Genetic Technology News, 1986

[a]'Genetic' refers t. single gene disorders (e.g., cystic fibrosis, sickle cell anemia) and chromosomal disorders (e.g., Down syndrome). 'Genetic predispositions' refer to common disorders known to have a genetic component such as heart disease and diabetes,

SOURCE: Office of Technology Assessment, 1988

cer, diabetes, or heart disease, they would appear to be of considerable interest to insurers. However, it is important to understand some of the technical characteristics and limitations of these tests before concluding that they will be adopted by insurers or employers.

Tests for genetic disorders have, to date, been used almost exclusively within the disciplines of pediatrics and obstetrics. For example, in all States, newborns are screened for one or more genetic conditions amenable to effective treatment (e.g., phenylketonuria), and pregnant women 35 and older are routinely offered prenatal diagnosis to detect fetal chromosomal abnormalities such as those associated with Down's Syndrome. Aside from pediatric and obstetric applications, genetic testing has not yet become widely incorporated into the practice of medicine. Genetic screening programs targeted at young adults have generally involved specific racial and/or ethnic groups and have usually addressed reproductive risk rather than the presence of disease itself. For example, community-wide programs to identify carriers of sickle-cell disease or Tay-Sachs disease have been implemented to identify those couples who might benefit from genetic counseling and prenatal diagnosis.

Most available tests for genetic conditions are not based on recombinant DNA techniques. In fact, until recently, three basic approaches have been used to diagnose genetic conditions. First, chromosomal analyses are employed to detect conditions such as Down's syndrome. Tests for chromosomal abnormalities can be conducted with blood, with the fetal cells contained in amniotic fluid, or more recently, with chorionic villus samples (obtained during the first trimester of pregnancy). Second, biochemical assays to identify abnormal gene products or the consequences of abnormal gene function have been used. In the case of Tay-Sachs disease, for example, reduced activity of the enzyme, Hexosaminidase A, signals either the disease or carrier state. Lastly, genetic testing has relied on identifying clinical manifestations of the disease itself. Table 4-9 summarizes information on some common genetic disorders.

Most conventional genetic tests rely on detecting the products expressed by abnormal genes. As the gene product associated with most genetic disorders is unknown, there are relatively few genetic tests available. In some cases, the development of tests has been stymied by the inability to access tissues in which gene product abnormalities may be found (e.g., brain, eye). Many available tests are also of limited use, because irreversible damage may have already occurred by the time an abnormality in gene function is detected.

Since the 1970s, a variety of techniques has been developed that allow a more direct examination of the genes themselves. DNA-based tests overcome many of the limitations of conventional tests for genetic disorders. They can be of diagnostic use without knowing the gene's product or function; furthermore, because genes are present in virtually all body cells, tests can be applied using easily accessible tissues such as white blood cells, and in the case of prenatal diagnosis, fetal cells can be obtained through amniocentesis or chorionic villus biopsy. With an individual's genetic

Table 4-9.—Common Genetic Disorders

Genetic disease	Cause	Nature of illness	incidence	Inheritance
Down syndrome	autosomal chromosome abnormality	range of mental retardation	1 in 800	sporadic
Klinefelter's Syndrome	sex chromosome abnormality	defect in sexual differentiation	1 in 2,000	sporadic
Cystic fibrosis	?	complications of excessively thick mucus secretion	1 in 2,000 Caucasians	autosomal recessive
Huntington's disease	?	progressive mental and neurological degeneration	1 in 2,500	autosomal dominant
Duchenne muscular dystrophy	?	muscular degeneration, weakness	1 in 7,000	X-linked
Sickle-cell disease	abnormal hemoglobin	impaired circulation, anemia, pain attacks	1 in 625 mostly black	autosomal recessive
Hemophilia	defect in blood clotting factors	uncontrolled bleeding	1 in 10,000	X-linked
Phenylketonuria	enzyme deficiency	mental deficiency	1 in 12,000 mostly Caucasians and Orientals	autosomal recessive
Tay-Sachs disease	absence of an enzyme	buildup of fatty deposits in brain, leading to early death	1 in 3,000 Ashkenazic Jews	autosomal recessive
Lesch-Nyhan syndrome	enzyme deficiency	mental retardation, self-mutilation	1 in 100,000	X-1inked

SOURCE: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of General Medical Sciences, "The New Human Genetics," Nlli Pub. No. 84-882 (Wash~ngton, DC: U.S. Government Printing Office, 1985).

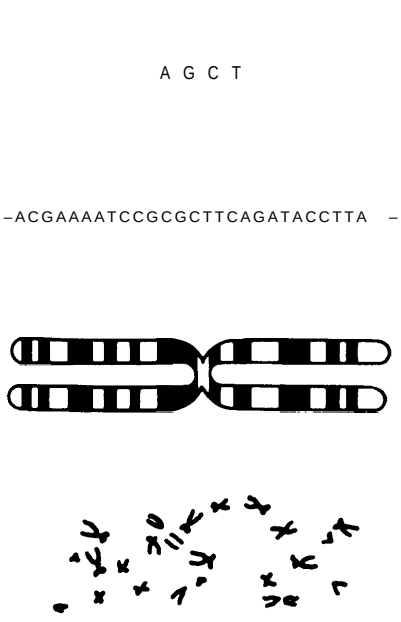
makeup determined at conception, DNA-based tests can be administered pre- or post-natally and before the onset of symptoms of the disease. Early diagnosis affords the possibility of therapeutic intervention to prevent manifestations of disease. As genes that are associated with an increased risk of common disorders such as heart disease and diabetes are identified, tests may be applied widely, perhaps as part of a population screening effort. Before describing the newer genetic diagnostic methods, a review of some basic genetic principles is in order.

Some Basic Genetic Principles: A Review

Genes are lengths of deoxyribonucleic acid (DNA) that have three main functions: 1) they code for polypeptide chains, the components of proteins; 2) they have important regulatory functions; and 3) they self-replicate during cell division. The human genome contains an estimated 100,000 genes arranged along the length of chromosomes (figure 4-4). DNA is a macromolecule made up of two chains containing four nucleotide bases—adenine, guanine, cytosine, and thymine or A, G, C, and T. The two chains are complementary. The adenine base on one chain will always bind with the thymine base on the other, and cytosine on one chain always binds with guanine on the other. Hydrogen bonds hold the bases of the two chains together to form a spiraling helix (the double helix). To illustrate the concept of the complementary nature of the two chains, if one strand had the base arrangement of CCAT, its complementary strand would be GGTA (figure 4-5).

The location of a gene along the length of DNA is called its locus. Because chromosomes occur in pairs (humans have 23 pairs), there are two copies of a gene at each locus, one inherited from each parent. Different "versions" of a gene at a particular locus are called ***alleles.*** When there are two or more versions (alleles) of a gene at a par-

Figure 4-4.–Organizational Hierarchy of DNA, the Carrier of Genetic Information in Human Cells



Nucleotides

Adenine, guanlne, cytosine, and thymine, the basic building blocks of DNA.

Genes

Functional units of DNA needed to synthesize proteins or regulate cell function.

Chromosome

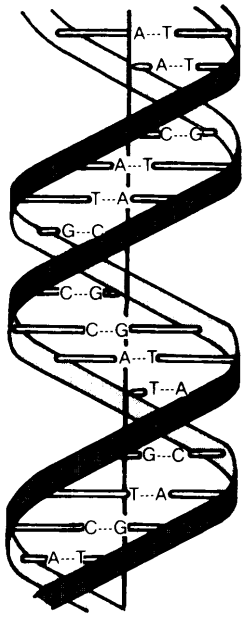
Thousands of genes arranged in a linear sequence, consisting of a complex of DNA and proteins.

Genome

The complete set of genetic information; each human reproductive cell contains 23 chromosomes, and all other **cells in the body contain a full set of 46 chromosomes.**

SOURCE: Office of Technology Assessment, 1987,

Figure 4-5. —A Schematic Diagram of the DNA Double Helix



SOURCE: Office of Technology Assessment, 1987

ticular locus and the allele has a frequency of at least 1 percent in the population, the genetic variant is referred to as a polymorphism.

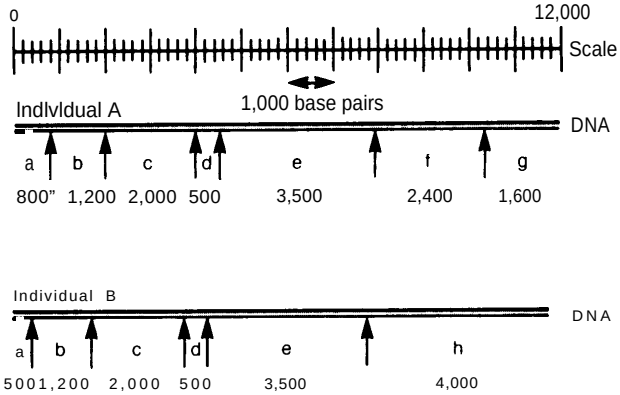
Given the variety in human characteristics, it is not surprising that there is variability in the DNA sequence. Some of the variability is significant in that a change in the expression of the gene results. In some cases, this change causes disease; in other cases, no disease. Other variations in genes occur but have no effect on gene expression.

A number of techniques has been developed that help identify genetic variation at the DNA level and consequently assists in distinguishing between those with disease-causing alleles from those with normal alleles. There are two basic approaches. In the Zinkage method DNA markers associated with abnormal alleles within families are used to predict family members' risks. Second, when the disease-causing alleles have been identified, direct genetic tests can be used.

Linkage methods.—The discovery of enzymes called "restriction enzymes" that cut DNA at specific sites has contributed to the development of linkage tests. This method exploits the variation that occurs along the length of DNA. The action of the restriction enzymes is sometimes affected by this variability. For example the restriction enzyme EcoRI cuts at the base sequence GAATTC on one DNA strand and at CTTAAG on the other. Following the action of this restriction enzyme, the sequence AATGAA TTCGT would be cleaved into two segments of DNA. If, however, an individual had a different sequence at the recognition site, say AATAAA TTCGT, the restriction enzyme would not cleave the DNA and there would be one long sequence. These differences in DNA fragment length after subjecting DNA to the action of restriction enzymes are called rest~"ction ***fragment length polymorphisms*** or ***RFLPs*** (figure 4-6). More recently, synthetic DNA cutters have been made that will enable researchers to cleave DNA anywhere along its length (272).

Investigators have studied the "inheritance" of RFLPs in families in which a genetic disease occurs. Once it has been established that a particular RFLP is almost always present in individuals with a disease and is almost always absent in those without the disease, the RFLP can be used as a

Figure 4-6.—Restriction Fragment Length Polymorphism (RFLP)



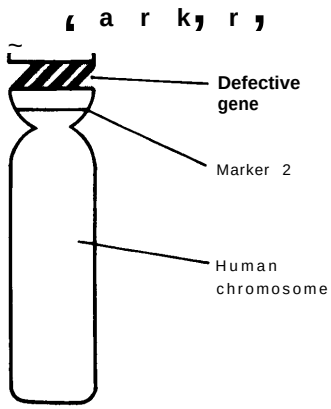
When a specific restriction enzyme cuts DNA, it may produce fragments of different sizes in the DNA of different people.

SOURCE U S Department of Health and Human Services, Publlc Health Service, National Institutes of Health, National Institute of General Medtcal Sciences, The New Human Genetic s," N IH Pub No 84.662 (Washington, DC U S Government Prlntlng Off lce, 1985)

marker for that disease (different polymorphisms may be associated with the same disease in different families). Such RFLP disease markers are in close physical proximity along the length of DNA to the disease-causing gene (figure 4-7). How linkage analysis works is well illustrated in the case of Huntington's disease (figure 4-8).

In order to conduct linkage analyses, geneticists must usually have blood samples from more than one affected family member. In addition, sometimes at least two generations of family members must be tested. Once blood samples are obtained, DNA must be purified from white blood cells. Next, a technique called Southern blotting is used to visualize individual RFLP patterns (figure 4-9). First, the DNA is cut into fragments with restriction endonucleases. The fragments resulting from the digestion are then placed on a gel and a charge is applied (a process called electrophoresis) (figure 4-10). Because of their different sizes, the fragments migrate along the gel at different speeds and segregate. Labeled probes are prepared (copies of the different RFLPs are made and labeled) and applied to the gel. Both the DNA fragments and probe are "denatured," making them single-stranded. The single-stranded probe then binds to (hybridizes with) complementary DNA sequences. Because the probe is labeled (either radioactively or with Biotin, a nonradioactive chemical), distinct bands representing the fragments of different lengths can be visualized.

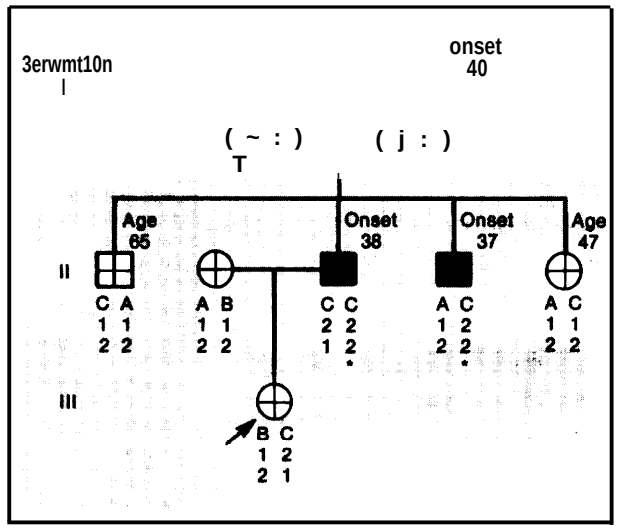
Figure 4.7.—Genetic Linkage



Discovering specific DNA markers near a defective gene greatly improves the accuracy of diagnosing the genetic disorder.

SOURCE: Integrated Genetics Inc., "Annual Report 1985," Framingham, MA, 1986.

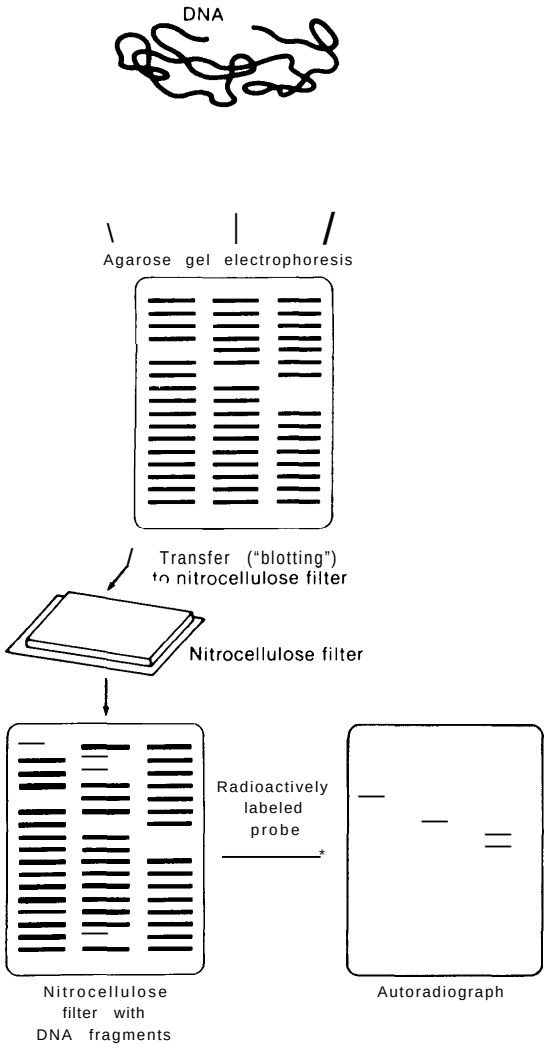
Figure 4.8.— Linkage Analysis for Huntington's Disease (HD)



Thl.S tlgure presents the result of a genetic iinka~e test Using three different restriction enzymes. The first p~oduces fou~ possible RFLP's listed as A, B, C, or D: the second and third enzymes produce two RFLP's listed as 1 or 2. The three RFLPs for each of the two number 4 chromosomes are presented vertically under each individual. In this example an at risk consultant had an affected father and uncle and an affected grandmother who is deceased. The form of the RFLP linked to the HD gene appears to be C22 since this is the RFLP that the father and uncle have in common. The consultant had inherited a B12 from her mother and a C21 from her father and therefore has probably not inherited the HD gene.

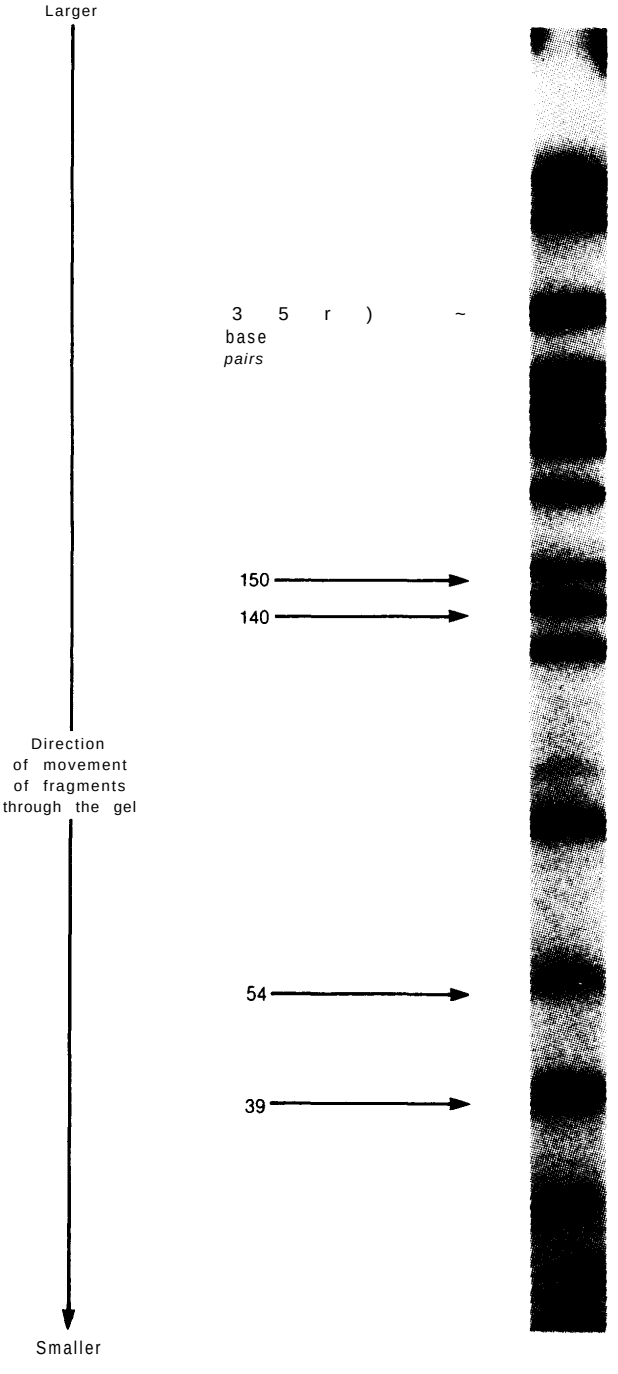
SOURCE: R. Myers, "Genetic Linkage Test for Huntington's Disease," The Genetic *Resource* 4(1):15-18, 1987.

Figure 4-9.—Southern Blotting



SOURCE U S Department of Health and Human Serwces, Public Health Serv. Ice, National Institutes of Health. National Institute of General Medl. cal Sciences, "The New Human Genetics, " NIH Pub No 64-662 (Wash Ington, DC. U S *Government* Printing Office, 1985)

Figure 4-10.— Electropheresis Preparation of a Southern Blot



SOURCE U S Department of Health and Human Ser-wces, Public Health Serv. Ice, National Institutes of Health, National Institute of General Medl. cat Sciences, "The New Human Genetics, " NIH Pub. No. 84-662 (Wash. tngton, DC: U S Government Print~ng Of ftce, 1985),

The use of linkage tests is limited, because the exact location of the deleterious gene is not known. Instead, an analysis of the transmission of linked markers within families in which the disease occurs forms the basis of the test. Because these analyses require the cooperation of multiple family members, they are not widely applicable. When affected family members are deceased, or when communication regarding the disease is poor, such testing efforts can be hampered.

Because linkage analyses track the inheritance of a marker close to the disease-causing allele, it

is vitally important to accurately categorize those represented in the family tree (pedigree) as affected or unaffected. Sometimes the diagnosis of genetic conditions such as Huntington's disease, for which no definitive diagnostic test is available, are subject to error. Alcoholism, multiple sclerosis, and a number of other necrologic disorders have been misdiagnosed as Huntington's disease. In addition to ensuring that family members participating in linkage analysis are correctly identified as affected or unaffected, sometimes evidence of paternity is sought for those participating in the family studies to further guarantee the accuracy of the tests.

Even when the appropriate family members are available and the diagnosis of the genetic condition is well established, linkage tests may not be informative. For the tests to be informative, family members must be polymorphic (i. e., have different versions of the allele) at the relevant restriction site(s). In the case of Huntington's disease, some families cannot benefit from linkage analyses because they lack "heterogeneity" for the RFLP. That is, those with the disease have the same base pairs at the restriction site close to the deleterious gene as unaffected members. Even for those families that show variation and can be studied using linkage analyses, the risk of being affected for any particular family member (or fetus in the case of prenatal diagnosis) is rarely given as zero or 100 percent. The certainty with which diagnoses are made depends on how tightly linked the marker is to the disease-causing allele.

As the distance between the marker and the deleterious gene increases, the chance of an exchange of DNA between chromosomes (called "crossing over") within this region during meiosis (the cell division occurring at the time of conception) increases. If the exchange took place between the marker and the disease-causing allele, an erroneous linkage study result would occur. Diagnostic certainty increases when there are two RFLP markers flanking the deleterious gene. Flanking markers have been identified for cystic fibrosis, so when a family undergoes linkage testing, the chance that the results represent their true genetic state is very high. Sometimes, the RFLP and the gene are observed to be "very tightly linked." When all of those with disease have one form of an RFLP and those without disease have another form, the association suggests that the polymorphic restriction cleavage site includes the disease-causing gene. Such tightly linked polymorphisms have been observed for phenylketonuria (PKU) and some thalassemias (table 4-10). The specific location of the gene that causes cystic fibrosis has recently been identified (326). When verified, a direct test will become feasible.

For some disorders, spontaneous mutation accounts for a relatively large proportion of cases. For example, in the case of Duchenne Muscular Dystrophy, even though flanking markers for the gene have been located, linkage tests have been reported to be uninformative in many families studied. Some of the inconclusive results occur because an estimated 10 to 15 percent of cases of the disease result from spontaneous mutations that are not inherited.

In summary, linkage studies are unwieldy because they involve multiple family members, are technically difficult to conduct and therefore expensive ($500 to $1,000), and their interpretation is somewhat subjective and requires a great deal of knowledge regarding the expression of the dis-

Table 4-10.—Available Genetic Tests by Type of Test[a]

Test type	Disease
Linkage RFLP tests:	Becker's muscular dystrophy
	Carbamyl phosphate synthetase deficiency
	Chronic granulomatous disease
	Cystic fibrosis
	Fragile X syndrome
	Hemophilia A and B
	Huntington's disease
	Myotonic dystrophy
	Neurofibromatosis
	Polycystic kidney disease (adult)
Direct tests:	Alpha, antitrypsin deficiency
	Duchenne muscular dystrophy
	Growth hormone deficiency
	Lesch-Nyhan disease
	Ornithine transcarbamyiase (OTC) deficiency
	Retinoblastoma
	Sickle cell anemia
	Thalassemia (some forms)
Tests for very tightly linked polymorphisms:	Hemoglobinopathies
	Phenylketonuria (PKU)
	Thalassemias

aThis represents a selected list of tests available as Of early 1988,

SOURCE: Office of Technology Assessment, 1988,

order in question. However, with the rapid discovery of more genetic markers, linkage studies will be available for an increasing number of disorders. (Genetic maps have been constructed that already include the relative positions of more than 400 markers (69).)

Direct Genetic Tests.—When a disease-causing gene has been identified, direct tests have been developed that avoid many of the problems associated with linkage analyses. Direct tests do not rely on the analysis of multiple family members and therefore may be amenable to population-wide screening. To date, there are few conditions for which these direct tests are available (see table 4-10), and with the exception of sickle cell anemia, the conditions are rare.

The "candidate gene" method can be used to identify disease-causing genes when the product (a protein) of the gene is known or suspected. By working backwards from the gene product, a strand of DNA that is complementary to the gene of interest is created. This is accomplished by taking the messenger RNA (genetic material involved in protein synthesis) for the protein and using the reverse transcriptase enzyme to manufacture a complementary strand of DNA (called cDNA). cDNA can be made for both the normal and abnormal gene products. Once the cDNA has been shown to accurately distinguish affected from unaffected individuals, it can be used as a diagnostic test.

If a disease-causing gene has been located and sequenced, then specific gene probes, complementary to the abnormal gene, can be synthesized, labeled, and used to conduct direct tests using the Southern blot technique. Gene probe tests are available for sickIe cell anemia, retinoblastoma, and some forms of thalassemia (see table 4-10).

Three techniques have been developed that will allow the detection of any genetic variation along a particular DNA sequence. The first method relies on the synthesis of short segments of DNA sequences called "oligonucleotides. " In order to synthesize oligonucleotides, the amino acid sequence of at least a part of the gene product must be known (if known, the related DNA sequence can be deduced). Testing conditions are manipulated so that these oligonucleotide probes, when applied to a Southern blot, only bind to perfectly complementary sequences. Through comparisons with a sequence of normal DNA, any number of abnormal ,alleles at a particular locus can be identified (156). A second technique relies on detecting mismatches between a radioactively-labeled RNA probe and an individual's single-stranded DNA. If the DNA includes an abnormal allele, it will not match perfectly to the RNA probe. When the resulting hybrid is mismatched, the enzyme, ribonuclease A, will cut the RNA probe at the site of the abnormal allele (211). The third technique detects differences in the paired probe and DNA sequence according to their migration on a "denaturing gradient gel. " A perfectly matched pair of probe and DNA sequence will migrate on a gel differently than a mismatched pair (90).

Until recently, one technical difficulty limiting the use of direct genetic tests was the inability to obtain adequate amounts of DNA, especially when analyzing prenatal diagnostic specimens. *A* procedure to produce additional DNA from a patient sample has recently been developed (157, 255). After a sample is obtained, an enzyme (DNA polymerase) can be used to amplify targeted DNA sequences more than 200,000-fold. Improvements in this technique have allowed direct visualization of the DNA fragments of interest on standard gels without the use of radio-labeled probes (227). These advances simplify and accelerate the diagnostic process and once automated, will allow more laboratories to conduct DNA-based genetic tests.

Limitations of Genetic Tests

Although direct genetic tests avoid many of the problems inherent in linkage analyses, both methods may yield inaccurate results because of some unique characteristics of genetic diseases. Some genetic conditions can be caused by more than one mutation, either at the same or at a different locus. For example, G6PD (glucose-6-phosphate dehydrogenase) deficiency may be caused by different mutations at the same locus. In the case of congenital deafness, different recessive genes at different loci can lead to the same clinical picture. And at least two different loci have been

implicated in families in which familial manic depression occurs (79,136). Unless such heterogeneity is identified, both linkage-based and some of the direct tests may be misleading.

Another factor that may lead to diagnostic confusion is the extent to which a deleterious gene is expressed, and when expressed, the constancy of its expression. Sometimes, individuals with a genotype associated with disease do not express the disorder at all. Such a condition is said to have reduced "penetrance. " For example, retinoblastoma, a dominantly inherited eye cancer, has reduced penetrance. Before an individual who has inherited the abnormal allele expresses it, a mutation must occur in the cells of the eye. Furthermore, some conditions, although expressed, are variable in their expression. Some individuals may be mildly affected, while others are severely affected. In the case of tuberous sclerosis, although the majority of cases represent new mutations, there are cases in which a severely affected child inherited the gene from a very mildly affected parent in whom the disorder had not been recognized.

Prospects for Genetic Testing

As of the end of 1987, there were no FDA-approved recombinant DNA tests for human genetic conditions (325). Instead, a limited number of tests were available through university genetics programs or through a few commercial laboratories. According to a 1986 survey of biotechnology companies, eight companies plan to offer genetic tests as a laboratory service, and six plan to have diagnostic test kits ready for sale by 1991 (291). One company is in the process of evaluating the predictive value of markers (RFLPs) in detecting an individual's susceptibility to atherosclerosis and hypertension. Cetus Corporation has developed an automated method to amplify DNA and conduct genetic analyses without radioisotopes (82).

Available DNA-based tests for genetic disorders are technically difficult to perform and most rely on the conduct of family studies. As more direct tests become available and as the technologies are simplified, genetic testing may become a part of routine care. However, given the nature of genetic diseases, there will be a significant element of diagnostic uncertainty associated with the tests for many conditions. Heterogeneity, reduced penetrance, and variable expression of some genetic conditions will make predictions difficult based on the results of genetic testing. Furthermore, tests for common disorders with a genetic component (e.g., heart disease, cancers) will rarely be definitively diagnostic. Instead, a positive result from a genetic test would usually mean that an individual's relative risk (relative to those without the gene) is increased. In some cases, modification of lifestyle (cessation of smoking, changes in diet) may reduce the relative risk substantially. In other cases, early medical interventions may alleviate the increased risk.

If genetic tests can provide information that would lead to the adoption of preventive interventions, they may be embraced by primary care physicians. In the past however, primary care physicians have not adopted new genetic tests (51). This may be attributed to the fact that to date, tests have been used primarily to provide reproductive risk information to couples, rather than information regarding the health status of the individuals themselves. Primary care physicians have little exposure to genetics in medical school (47), and there have been few continuing education opportunities directed at the community-based physician. Furthermore, there are few genetic specialists available to offer genetic testing and counseling on a consultative basis (137).

For many diseases there will be a lag period between the time genetic tests are available and the time when effective interventions for the diagnosed condition are available. Other than for reproductive planning, individuals may not find the tests useful. In the case of a debilitating, late-onset condition, however, having risk information early in life may be helpful in making employment and other life decisions. How readily such tests will be adopted remains to be seen. In the case of Huntington's disease, before the announcement of a predictive test, approximately 55 to 80 percent of those at risk indicated that they would elect to be tested when the test became available (258, 275, 285). However, although interest in testing remained high after the announcement of the predictive test (174), in the first several months of testing, less than 15 percent (44/349) of those informed of the test's availability by one of three

genetic centers offering the test have requested testing (236).

Will genetic tests be used in underwriting or as part of an employee applicant screening program? The answer depends on many factors. Genetic tests in their present state are impractical to administer, require considerable technical skill both in their conduct and in their interpretation, may require analyses of multiple family members (i.e., linkage-based studies) that would be unacceptably intrusive, and are very expensive to conduct. (A direct test for sickle cell disease may cost as much as $450, and linkage-based tests as much as $1,000.) Once tests are simplified and less costly, and when direct tests for predispositions to common diseases become available, they may be considered attractive to insurers in evaluating an applicant's risk.

Although commercial insurers may be disinclined to test for rare genetic conditions such as Huntington's disease, they would be interested in learning of the results of any genetic tests already conducted by the applicant's physician. Thus, for rare conditions, it appears that the impact of genetic tests on the underwriting process will be felt when genetic tests become a part of routine care.

The availability of genetic susceptibility tests may have a dramatic impact on who the insurer decides to test. At present, a small proportion of applicants are tested. In general these are individuals who indicate a history of disease or presence of a risk factor (e.g., age, hypertension) on the application. As genetic tests can indicate risk in the absence of clinical signs of disease, they may be applied to all age groups to identify risk regardless of medical history. If widely used by primary care physicians in their provision of preventive health care, insurers may be in the position of testing to avoid the prospects of adverse selection.

TESTS OF POTENTIAL INTEREST TO INSURERS BECAUSE OF DISEASE PREVALENCE

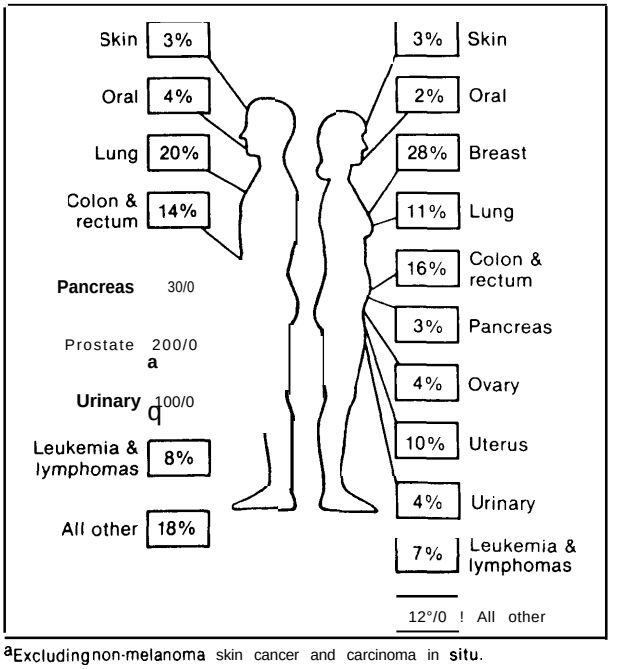
Testing for Cancer

Currently Available Cancer Screening Tests

Although employers and insurers deal with a relatively young and therefore low-risk population in terms of cancer incidence, a large number of cancers occur among those under 55. Nationally, approximately 19 percent of the estimated 930,000 new cases of cancer occurring in 1986 were diagnosed among those aged 15 to 54 (302, 293). Figure 4-11 summarizes 1988 estimates of the distribution of new cases of cancer by site and sex.

Screening tests for latent disease are available for several of the most common cancers (e.g., colon, breast, and uterine/cervical cancers). The American Cancer Society (ACS) has considered the epidemiologic data regarding the impact of screening tests on mortality and has made recommendations to physicians regarding the incorporation of screening into their practices (box 4-A). For example, mammography has been ***recommended*** for women 50 and older, based on observed mortality differences between groups of

Figure 4-11.—Distribution of New Cases of Cancer by Site and Sex[a]—1988 Estimates



[a]Excluding non-melanoma skin cancer and carcinoma in situ.

SOURCE American Cancer Society, Inc "Cancer Facts and Figures, 1987, " New York, NY, 1988

Box 4-A.—Screening Tests for Some Common Cancers

Disease: Colorectal cancer

Screening Methods:

Digital rectal examination: Although widely used as part of routine medical examinations, 90 percent of colorectal cancers are not palpable using the digital rectal examination.

Sigmoidoscopy: Using a rigid or flexible scope, physicians can examine from 30 to 60 cm of the colorectum. The flexible scope is more comfortable for the patient but is more difficult for physicians to use. Given the distribution of neoplasms, the flexible scope will miss about **40** percent of cancers (111).

Stool ***occult blood test:*** There are several commercial fecal occult blood tests available. The most widely used is the Hemoccult II® screening test. The examination of two samples is recommended from each of three consecutive bowel movements while an individual is on a meat-free, high-roughage diet. Individuals are also advised to avoid use of aspirin, nonsteroidal anti-inflammatory drugs, vitamin C, iron, laxatives, rare red meat and fruits and vegetables high in peroxidases (e.g., turnip, cauliflower). Even when used optimally, Hemoccult II® misses as many as 10 percent to 30 percent of cancers and 65 percent to **75** percent of colonic polyps (29). Initial reports on HemoQuant™ a recently introduced quantitative test for occult gastrointestinal bleeding, suggest it is more sensitive than Hemoccult, However, increased detection of upper gastrointestinal tract bleeding and dietary hemoglobin may reduce its specificity (153). Several new methods are being developed that will be more sensitive and specific than current screening methods. For example, immunoassay are being developed that will limit cross reactivity with foodstuffs, animal hemoglobins, and drugs.

Screening recommendations:

American Cancer Society: Patients over the age of **40** should have a digital rectal examination annually. T'hey should have a six-slide stool occult blood test annually after the age of **50** years. After age *50,* ~igmoidoscopy should be done annually for 2 years, then every 3 years.

Canadian *Task Force:* Patients should have an annual stool occult blood test starting at the age of **16** years.

Epicfemiologic data in support of recommendations: Two prospective controlled trials are ongoing to assess the impact of stool occult blood testing on mortality (106,320). Preliminary data indicate that occult blood testing confers an advantage in detecting localized cancers over sigmoidoscopy alone or usual care.

Disease: Breast cancer

Screening Methods:

Mammography. The most widely used breast imaging techniques are screen-film mammography and xeromammography. Preliminary results of the Canadian National Breast Screening study show first screen mammography to have a sensitivity of **69** percent, a specificity of **94** percent, a positive predictive value of 8.6 percent and a negative predictive value of 99.7 percent (13).

Screening recommendations:

American Cancer Society: All women should: 1) do monthly breast self-examinations, **2)** have a physician perform a breast examination every 3 years between the ages of **20** and 40 years and annually thereafter, and 3) have a mammogram for baseline purposes between the ages of 35 and **40 years, every 1** to 2 years between the ages of **40 and 50 years, and annually thereafter.**

U.S. ***Preventive Services Task Force:*** Women **50** years of age and older should be offered annual clinical breast examinations and mammography. For women at high risk, especially those with a family history ~f premenopausally diagnosed breast cancer in first-degree relatives, physicians may elect to recommend mnual clinical breast examination and mammography beginning at an earlier age (e.g., 35 years) (314).

Canadian Task Force: All women should have an annual physician breast examination and mammography between the ages of 50 and 59 years.

Epidemiologic data h support of recommendations: Two randomized controlled trials have shown reductions in breast cancer mortality secondary to breast cancer screening. The results of the Health Insurance Plan (HIP) of Greater New york study show that yearly mammography, physician examination, and Patient self-examination reduced breast cancer mortality in the study groups by **30** percent for women over **50 268).** In Sweden, a **40** percent reduction in breast cancer mortality was demonstrated among screened women ages **50** to **74 years (282).** The benefits of screening for women under **50** should be determined by the National Study of Breast Cancer Screening, being conducted in Canada (results are not expected before the 1990s) (197).

Disease: Uterine cervical cancer

Screening Methods:

Papanicolaou smear or PAP test: Cells scraped from the cervical os are examined to identify cancerous and pre-cancerous cell morphology. The false negative rate associated with the PAP test is estimated to be about 30 percent (243).

Screening recommendations:

American Cancer Society: All women who are or have been sexually active, or who have reached age 18 should have an annual PAP test and pelvic examination. After a woman has had three or more consecutive, normal annual examinations, the PAP test may be performed less frequently at the discretion of her physician (318).

Canadian Task Force: Women between **18** and **35** years of age should have a PAP test annually. A PAP test should be done every five years between ages 35 and 70. No PAP tests are necessary after the age of **70** years.

Epidemiologic data in support of recommendations: Definitive evidence that PAP testing reduces mortality is not available. However, a body of inferential data supports the contribution of PAP testing to reduced mortality. The incidence of invasive cervical cancer is significantly lower among screened as compared to unscreened groups of women (88,170). Other evidence in support of the efficacy of PAP testing is that there are correlations between the proportion of women screened in an area and the cancer incidence and mortality rates of that area (.53). Lastly, the decline in cervical cancer incidence between the mid-1950s and mid-1970s is consistent with the adoption of PAP screening (118).

Disease: Lung cancer

Screening methods:

Chest *x-ray, sputum cytology:* Chest x-rays and sputum cytology are used to complement one another. Chest x-rays are useful in detecting peripheral tumors, whereas sputum cytology is used to detect centrally located tumors.

Screening recommendations:

American Cancer Society: Specific screening for lung cancer is not indicated.

Canadian Task Force: Same recommendation.

Epidemiologic data in support of recommendations: The National Cancer Institute's (NCI) Cooperative Early Lung Cancer Detection Program that was started in the early 1970s assessed the ability to improve lung cancer detection and lower lung cancer death rates in high-risk men (smokers age 45 and older) by adding sputum cytological screening to chest x-ray screening exams. When the NCI trials commenced, it was generally accepted that yearly chest x-rays were not effective in reducing lung cancer mortality and that a large proportion of cancers detected during the trials would be detected by sputum cytology. The results of the randomized controlled trials indicate that although lung cancer detection is somewhat improved with the addition of sputum cytology screening, there is no improvement in lung cancer mortality among those subjected to both screening methods. Contrary to expectations, the majority of lung cancers were detected radiologically and not cytologically (sputum cytology alone detects **15 to 20** percent of lung cancers, almost all of which are squamous cancers with a favorable prognosis) **(91,198). The** evaluation of x-ray itself as a screening methodology has not been possible, because annual chest x-rays have, in many areas, become a part of routine care.

women randomized into screened and not screened study populations (268). Similar trials are underway as part of an evaluation of screening tests for colon cancer (106,320).

Although effective in reducing mortality when applied to age-appropriate populations, most available screening tests are not highly sensitive or specific. In the case of colon cancer, the Hemoccult II® kit, the most widely used commercial kit to detect occult blood in the feces (stool) is estimated to detect only 25 to 35 percent of colonic polyps (an estimated 5 percent of polyps detected would develop into colorectal cancer if not removed (2)) and 70 to 90 percent of cancers (29). Because of the relatively low sensitivity, the American Cancer Society recommends that people over *50* have sigmoidoscopy performed every year for 2 years and then every 3 years, in addition to stool occult blood testing. Although the

specificity of Hemoccult II" is relatively high—97 percent for benign polyps and carcinoma—the predictive value of the test when applied to an asymptomatic population is relatively low. Fifty-two percent of positive tests represent cases of either polyps or cancer; 40 percent represent polyps, and 12 percent represent cancer (11). For every 1,000 individuals screened, there will be an estimated 20 to 60 positive results, of which about half would represent cases of polyps or cancer (figure 4-12). All positive cases need to be further evaluated by full colonoscopy or, less definitively, by flexible sigmoidoscopy or barium enema (29). Although the test for fecal occult blood is inexpensive to administer and interpret, the cost of evaluating a positive case may be as high as $1,000. In addition to being costly, the follow-up procedures are invasive and uncomfortable to the patient.

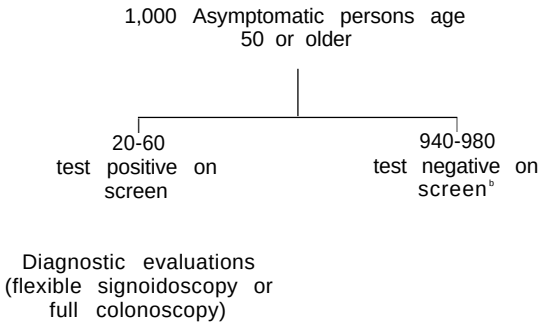
Perhaps because of its poor predictive value, physicians generally recommend stool sampling to their patients less often than published guidelines recommend. For example, despite the ACS recommendation for an annual screen for occult blood in the stool after age 50, surveys indicate that few in that age group (3 to 20 percent) have ever had one (284). Furthermore, in a 1984 survey of primary care physicians, only 48 percent reported that they followed or exceeded the ACS guideline for stool blood sampling with all patients (S). The low utilization of colorectal screening may also be explained by poor patient compliance. The screening test, which relies on stool sampling, is unacceptable to many individuals, and as many as 30 to 50 percent of patients given slides to return with stool specimens do not do so *(2, 321).* Both patient and physician compliance may be improved when results of the two randomized trials being conducted to assess the impact of fecal occult blood screening on mortality are completed. In addition, tests with higher sensitivity and self-tests have been developed, which may improve compliance.

Other currently available, recommended screening tests are also underutilized. Despite findings from randomized clinical trials showing reductions in mortality attributable to mammography screening for breast cancer, the procedure is underutilized according to ACS guidelines. Although the ACS recommends that women 50 and older have an annual mammogram, the 1984 survey of over 1,000 primary care physicians indicates that only 11 percent of physicians follow or exceed the ACS guidelines with all patients. In a study in which physician screening practices were compared with those expected based upon published guidelines, less than 10 percent of expected mammograms, given the age distribution of women seen in the outpatient clinic, were actually performed (322). Much of the disagreement with ACS recommendations stems from physician concerns over the exposure to x-rays associated with the procedure (5). Utilization may also be depressed due to the cost of the procedure. Mammography is relatively expensive and requires interpretation by a physician trained in radiology.

None of the widely available cancer screening methods is being directly used by insurers. These include tests for colorectal cancer, uterine/cervical cancer, and breast cancer. This may be explained by the tests' poor predictive values and ease-of-use, cost, and acceptability to the individual screened. Furthermore, as these screening tests are not widely used in medical practice, insurers are unlikely to obtain information regarding cancer screening test results when an attending physician is asked to document the applicant's medical history.

In contrast to the examples given above, despite the fact that there is no direct evidence that screening for lung cancer through chest x-rays decreases lung cancer mortality, chest x-rays continue to be widely used. Contrary to ACS guidelines, many physicians continue to conduct annual chest x-ray examinations on their patients who smoke. This may be explained by the fact that x-rays are a useful technique to detect lung cancers (as well as other diseases). Once detected, however, there are few effective treatments. This raises an important issue in screening. When screening tests are evaluated for their use by ACS and other professional groups, the focus is on the ability of

Figure 4-12.— Fecal Occult Blood Screening[a]



E

aAssumes protocol for stool sampling is followed.
bThe American Cancer Society recommends flexible sigmoidoscopy as a complementary screening procedure If conducted on those testing negative for blood in the stool (to 60 cm), 9%0 would be diagnosed with polyps and 10/. with cancer
cApproximately 75o/o of these would be colonic polyps and 259/o cancer

SOURCE: Office of Technology Assessment, 1988

the screening test to reduce mortality. In order to achieve significant mortality reductions, the cancer must be detected at a stage in which effective therapy is possible. From the point of view of those wishing to identify latent disease, with the intent of excluding individuals from insurance or employment, tests that are effective in detecting cancers, such as chest x-rays, may be useful.

Similarly, tumor marker assays have been used to detect cancers but usually identify them at an advanced stage, when they are not amenable to treatment. Consequently, tumor markers are limited in their applications. They are used to monitor cancer therapy, to classify and stage tumors, and, in some cases, to provide prognostic information (65).

Available marker assays often fail to distinguish nonmalignant from malignant disease. As the prevalence of nonmalignant disease greatly exceeds that of malignant disease, these tests are not very predictive when used as screening tests. Sensitivity may be high for advanced cancer but is usually less than *50* percent for early or localized cancer. For example, the carcinoembryonic antigen (CEA) test, the most widely used tumor marker available, is positive in more than *80* percent of stages C and D colon cancers (advanced stages), but is positive in less than *40* percent of stage A (early stage) colon cancers. First identified as a tumor marker in 1965, CEA is now known to be associated with colon, lung, breast, and pancreatic cancers. However, CEA may also be elevated in the presence of nonmalignant diseases such as hepatitis, ulcerative colitis, gastric ulcer, and renal disease. In addition, other factors such as age or cigarette smoking maybe associated with CEA elevations. When the test is applied to an asymptomatic population to detect cancer, positive tests are poorly predictive—in a population screening study of the CEA test, only 12 percent of positive tests represented CEA-associated cancers (55). Radioimmunoassay, enzyme immunoassay, and monoclinal antibody-based methods for measuring CEA are commercially available. Although test manufacturers do not recommend tumor marker assays for screening use, at least one life insurance underwriting publication has suggested that the CEA assay could be adapted and used to screen insurance applicants *(103).* However, because tumor markers such as CEA have not been used for screening purposes within the general medical community, they have not been used by health insurers to screen applicants.

Future Prospects for Cancer Screening

There have been reports of tests that may be more widely applied as screening tools for cancer. One such test is based on differences found between the lipid moieties of lipoprotein particles found in the plasma of patients with cancer (called oncolipids) as compared to those without cancer *(92, 328).* The differences, which may represent some type of host response to malignancy, can be detected using magnetic resonance imaging (MRI) (previously referred to as nuclear magnetic resonance (NMR) spectroscopy). Patients with cancers at a range of sites (breast, gastrointestinal tract, lymph nodes, lung, bone marrow, central nervous system, and genitourinary tract) have resonance spectra that differ from both those of healthy volunteers and those with nonneoplastic illnesses, such as benign tumors, end-stage renal

disease, myocardial infarction, sepsis, and diabetes mellitus. There are, however, two significant sources of false positive results—pregnant women and men with benign prostatic hyperplasia.

Although preliminary results from over *2,000* samples are promising (220), a prospective study needs to be conducted to determine how early this method can detect cancer development, and whether there is a change in result with therapy, remission, or relapse. If these studies indicate that the test may be applicable as a screening tool, the equipment, although expensive (current cost is about $500,000 each), will accommodate automated testing. More detailed analyses of the composition of all plasma lipids and the physical structures of the lipoprotein-lipid complex in various populations will improve the understanding of the underlying specific abnormality. Further research may lead to the development of a more direct assay that could be performed in laboratories without expensive MRI facilities (257).

There is a major difference in this MRI-based test and available tumor markers. Preliminary results indicate that there appears to be no correlation between the MRI spectrum observed and tumor histology or extent of disease. In contrast, tumor markers, such as CEA antigen levels, are correlated with type of tumor, histology, or tumor differentiation, as well as with the extent and sites of metastases (spread of the cancer beyond its original site). The MRI test, while potentially useful in screening for latent cancer, may not be useful in monitoring patient management. Furthermore, as cancer patients who were undergoing treatment or who had completed therapy have a range of MRI spectrum values that overlap with both normal persons and untreated cancer patients, any screening program using this technique would be expected to misclassify individuals with a recognized and successfully treated cancer.

Radioisotopes bound to antibodies specific for tumors are also being used to diagnose lung, breast, colorectal, ovarian, gastric, and pancreatic cancers. However, this in vivo imaging technique is not suitable as a screening technology (182).

It is widely recognized that genetics plays a role in the development of human cancers. For example, 30 to 40 percent of bilateral retinoblastoma (an eye cancer) cases are attributed to genetic factors. Approximately 10 percent of breast and colon cancers are genetic in origin (228). Guidelines have been established to help interpret family pedigrees to evaluate an individual's susceptibility to malignancy. For example, for those without cancer, the presence in the family of a first degree relative with cancers occurring in both of paired organs (not attributable to metastasis), multicentric tumors, or cancer that has occurred at an atypical age or at an atypical site, suggest a genetic predisposition to cancer (228).

Genes have been identified that promote carcinogenesis when they are inappropriately activated (oncogenes) or when they are inappropriately inactivated (anti-oncogenes). For example, ras oncogenes, when activated by point mutations, appear to precede the development of some colorectal cancers (27) and adenocarcinoma of the lung (247). Analysis of the DNA from tumor cells indicates that anti-oncogenes contribute to the development of retinoblastoma and lung cancer (158). Diagnostic tests based on recombinant DNA techniques (e.g., use of oligonucleotide probes) are proving to be useful in interpreting tumor pathology. Finding oncogenes in cancerous tissues is helping physicians decide how to manage patients with breast, lung, and cervical cancers, and neuroblastoma. Patients with many copies of an oncogene in their cancerous tissues appear to have a poorer prognosis than patients without oncogene amplification. However, this does not hold true for all tumors. Thus, some high risk cancer patients may be identified for particularly aggressive anti-cancer treatments. While presently a useful tool in cancer patient diagnosis and management, applications in screening have not yet been developed.

Investigators are searching for genetic markers of susceptibility to cancer. Using blood samples from individuals whose families are known to be at risk of developing cancer, one biotechnology company, with NCI support, is looking for the presence of DNA markers for defective genes associated with lung, breast, and colon cancer (185). Another biotechnology company has developed DNA probes to detect gene rearrangements associated with lymphoid malignancies and is also

evaluating a test for human papilloma virus, a virus associated with a high risk of cervial cancer in women (182).

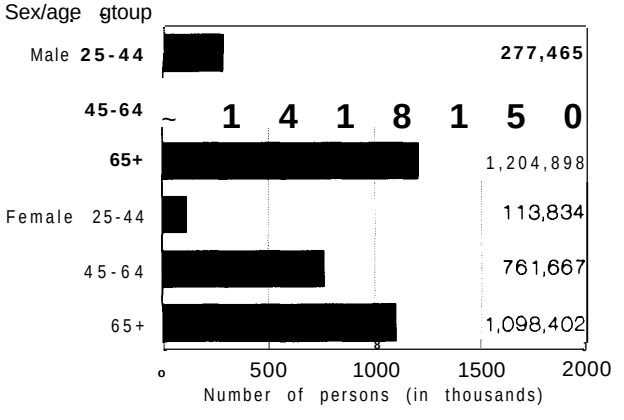
Testing for Heart Disease

Introduction

The prevalence of coronary heart disease (CHD, also called coronary artery disease or CAD) among those between the ages of 25-44 is estimated at 0.7 percent for males and 0.3 percent for females (8, 293). Figure 4-13 summarizes the prevalence of heart disease among persons age 25 or older. For males 15-44 years old, heart disease is the second leading cause of hospitalization, with an estimated 3.3 discharges per 1,000 males in that age group (295). Approximately 13 percent of heart attacks occur among males between the ages of 29-44 (8).

CHD age-specific mortality has been declining. Between *1972* and *1984* it declined by about 34 percent (7). Most of the decline has been attributed to changes in lifestyle, such as lowering dietary fat intake and stopping smoking. Improvements in managing hypertension and medical and surgical interventions for those with CHD have also contributed to the decline (108). Despite this decline in age-specific mortality, some predict that deaths, sickness, and costs associated with CHD could increase by more than 40 percent ***over*** the next quarter century. These increases are, in part, attributable to demographic trends and to increases in costs associated with CHD diagnostic technologies and treatments (301).

Figure 4-13.-Estimated Number of Persons With Coronary Heart Disease, 1985



SOURCE: Office of Technology Assessment, 1988.

Electrocardiogram (EKG) and Exercise Stress Testing

The electrocardiogram (EKG) is a record of the electrical activity of the heart and is used to detect abnormal cardiac rhythm and heart muscle damage. The EKG is not a sensitive test for presymptomatic heart disease *(93).* Exercise EKGs (also called stress EKGs) are more sensitive than resting EKGs. However, in a population at low risk for heart disease, the predictive value is poor. In a population with a prevalence of coronary disease of 1 percent, a person with a positive exercise EKG would have only a *7* percent chance of having CHD, while a person with a negative test would have a *0.3* percent chance of having CHD. While the relative risk is high (23 times the lower risk), the absolute risk is low (134). Exercise EKGs may be useful in evaluating a high-risk person who wishes to engage in strenuous physical activity, but it is a relatively expensive test that also carries a small, but definite risk (stress EKGs result in approximately 3 deaths per 10,000 patients).

An alternative to exercise EKGs will soon be marketed *(186).* A device called the ischemia scan uses *30* electrodes to measure approximately 500 heart beats for evaluation. The heart beats are analyzed by a high-speed array processing microcomputer, and the results indicate the overall amount of ischemic tissue (insufficient oxygen due to poor blood supply) present. The test is reportedly able to provide earlier detection of heart damage than EKGs, because it can test for as little as five grams of ischemic tissue, compared to an estimated 100 grams of tissue required to provide a positive reading with EKGs. The sensitivity is also reported to be higher than that of EKGs *(9o* percent versus 70 percent). Furthermore, the ischemia scan involves no risk of death, since no stress testing is involved. The scan's projected cost is comparable to that of the EKG (approximately $200). Additional testing is being conducted to evaluate the use of the scan among asymptomatic patients.

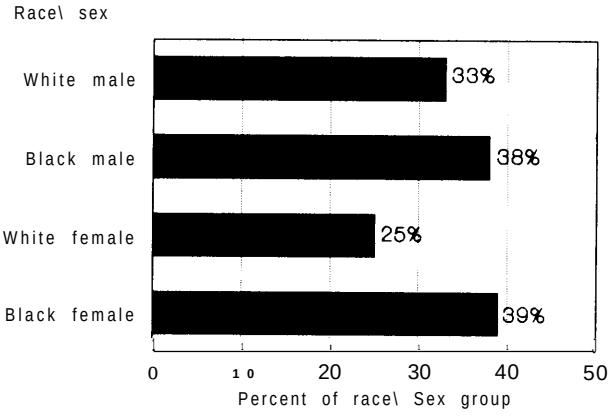
Coronary Heart Disease Risk Factors

Risk of future CHD disease can be determined by evaluating CHD risk factors singly or in combination. The three main predictors of CHD other than age and sex are hypertension, hypercholesterolemia, and cigarette smoking (181).

Hypertension. —Hypertension is a risk factor for heart attack, renal failure, stroke, and a number of other health problems (93). Blood pressure measurements are noninvasive, can be performed by a nonphysician, and are often performed during routine medical visits. According to medical practice guidelines, blood pressure should be recorded on any visit to a physician, not just at periodic health examinations (39). When hypertension is defined as a diastolic pressure greater than 95 mmHg, 38 percent of black males, 39 percent of black females, 33 percent of white males, and 25 percent of white females ages 18 to 74 are affected (figure 4-14). According to prospective studies, a 45-year-old hypertensive (systolic BP= 195 mmHg) male without other risk factors is at about twice the risk for CHD than a similar but normotensive male (7).

Although hypertension is a known risk factor for CHD, treatment of hypertension is not clearly associated with a decline in CHD deaths. One epidemiologic study (the Hypertension Detection and Follow-up Program) showed a 20 percent reduction in CHD deaths with control of hypertension, but other studies have not shown significant reductions of complications from CHD associated with hypertension control (93).

Figure 4-14.-Hypertension Prevalence by Sex and Race, U.S. Adults Age 18-74'



aH$_{ype}$~$_{en}$Si$_{ve}$S are defined aa persons with **a systolic level** 9reater than or Wual to 140 andlor a diastolic level of greater than or equal to 90 or who report using anti-hypertensive medication.

SOURCE: American Heart Associaticm, "1988 Heart Facts)" Dallas, TX, 1988.

Hypercholesterolemia. —Epidemiologic studies have shown a close relationship between serum total cholesterol and the subsequent development of CHD (154). The risk is not confined to those with extremely high values. Instead, the risk rises continuously with cholesterol level (273). An expert panel convened by the National Heart, Lung and Blood Institute has recently issued a report (3o3) setting a new standard for measuring cholesterol in adults over age 20. Previously, cholesterol levels above either the 90th or 95th percentile for age had been considered abnormal. The new classification specifies three total cholesterol ranges, characterizing those with high levels (240 mg/dl or more), borderline levels *(200-239* mg/all), and desirable levels (below *200* mg/dl).

As measurements of cholesterol, lipoproteins, and the protein components of lipoproteins are used in the evaluation of CHD risk, a brief description of cholesterol and its metabolism is in order.

Cholesterol is essential to the synthesis of cell membranes, steroid hormones (e.g., testosterone, estrogen), and is a component of bile (digestive juices). Cholesterol found in the plasma does not occur in a free state but is "packaged" and transported in the blood by plasma lipoproteins. Lipoprotein receptors are located on cell surfaces. Through these receptors, the cell binds to the lipoprotein containing the transported cholesterol. The cell then engulfs the lipoprotein (endocytosis), and the cholesterol is "carried" into the cell. This process, when functioning normally, keeps the blood concentration of cholesterol low enough to prevent the buildup of atherosclerotic plaques.

The plasma lipoproteins that carry cholesterol can be distinguished by their density. The three most important cholesterol-bearing lipoproteins are very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Lipoproteins differ in density because they consist of different proportions of lipids

(triglycerides and cholesterol). The LDL fraction carries 70 percent of serum cholesterol, compared to 20 percent carried by HDL (245). The protein components of lipoproteins are called apolipoproteins (13 major human apolipoproteins, A through H, have been described *(32)*). Apolipoprotein A (apo A) is the protein component of HDL, while apolipoprotein B (apo B) is the major protein component of LDL. These proteins exist in various forms. For example, both apo A and apo B exist primarily in two forms (apo A-I and A-II; apo B-1OO and B-48). Changes in serum levels of apo A and apo B correlate with changes in HDL and LDL, respectively.

Elevations in total serum cholesterol, LDL, and apo B are risk factors for atherosclerosis and CHD. Conversely, low levels of HDL and apo A are associated with CHD (43). Evaluations of the relative utility of plasma levels of cholesterol, triglycerides, HDL-cholesterol, and apolipoproteins in distinguishing healthy men from those with CHD have shown that levels of apo A-I, A-II and B are the best discriminators. In one study, 75 percent of men who were either normal or had significant coronary artery disease could be correctly identified using age and apo A-I, apo A-II, and apo B levels (160).

Much information about CHD risks has been gleaned from the Framingham prospective study, in which risk factors and heart disease status have been monitored for a cohort of initially asymptomatic individuals for more than 20 years. This study revealed that most cases of CHD arise from those in the population with only modest elevations of serum cholesterol. The average cholesterol level among those under age 50 who later developed CHD was only 244 mg/dl (154). Although high (NHLB1 defines abnormally high as 240 mg/dl or more), the fact that 244 mg/dl is the average indicates that many had levels in the borderline or normal range.

The strength of the association between cholesterol levels and CHD varies according to age and the presence of other CHD risk factors. The association declines with advanced age. In fact, total cholesterol is no longer predictive of CHD among men over age 65. The same cholesterol level may confer a different risk according to the presence of other risk factors (e.g., smoking, hypertension, abnormal glucose tolerance test). The relative risk of CHD developing within 18 years for a 35-year-old male with one risk factor–high cholesterol (total cholesterol of 335 mg/dl)—as compared to a 35-year-old male with no risk factors, is 3.9. The relative risk increases to 23.2 when both cholesterol and blood pressure (BP) are elevated (systolic BP =195 mmHg) and to 60.2 when all risk factors are present (i.e., elevated cholesterol, elevated BP, smoking, abnormal glucose tolerance test, left ventricular hypertrophy) (154).

There has been concern over the accuracy of the estimated 100 million cholesterol tests performed yearly. A 1985 study of the reliability of cholesterol testing conducted by the College of American Pathologists (CAP) showed that many tests being conducted are inaccurate. As a part of their evaluation, about 5000 of the nation's top laboratories were sent identical samples. Nearly half of the results were, according to experts, unacceptable (26). In response to these problems, CAP has recently made available "certified reference materials" in the form of freeze-dried human blood serum. The freeze-dried samples can be reconstituted and tested along with other samples. These reference materials will help to ensure that instrumentation is properly calibrated. CAP also conducts a laboratory proficiency survey program on a quarterly basis (5o).

To achieve accuracy and reliability in cholesterol testing, a NIH panel (Laboratory Standardization Panel) has recommended that uniform cholesterol cutoff points be adopted to identify adults at high risk for CHD and that cholesterol measurements be standardized and that deviations from true cholesterol values not exceed 5 percent (within 5 years the deviation should not exceed 3 percent) (319).

Automated laboratory desk-top analyzers hold promise in facilitating mass-screening efforts. These new analyzers require only a small amount of blood per test and are inexpensive (less than $3 per screen) (112). Eleven lipid research clinics have evaluated a rapid, desk-top analyzer. The assay was applied to nearly 13,000 people at schools, work sites, shopping malls, and other

locations. The analyzer determines cholesterol levels within 3 minutes, giving results that vary about 1 to 4 percent from rigorous, standardized laboratory methods (78). Despite these favorable results, there is concern that results may not be accurate if machines are not maintained, as may be the case in nonmedical settings. The NIH Laboratory Standardization Panel has not recommended the use of portable chemistry analyzers to measure cholesterol, arguing that their accuracy has not been thoroughly evaluated and that staff are inadequately trained to use them.

Although widespread screening is now being encouraged by the NIH National Cholesterol Education Program, physicians appear to be reluctant to participate *(263).* This may in part be explained by some uncertainty regarding the beneficial effects of lowering cholesterol levels. Hypercholesterolemia is a recognized risk factor for CHD, and dietary and drug-induced reductions in serum cholesterol have been associated with fewer new cases of CHD among asymptomatic men with high levels (225). The Lipid Research Clinics Coronary Primary Prevention Trial, for example, showed that lowering LDL-cholesterol by 12.6 percent with medications was associated with a 19 percent reduction in CHD. However, in all of the studies, the decrease in CHD was offset by an increase in non-cardiovascular mortality, and in several of the studies, there were no differences in total morta]ity (225).

With continued medical education, physician screening practices may change. According to a 1986 national survey of physicians, *64* percent of physicians thought that reducing high blood cholesterol levels would have a large effect on heart disease, up considerably from 39 percent in 1983 (261). There is evidence that more individuals are having their cholesterol measured. According to a national survey, 46 percent of adults reported that they had their cholesterol level checked in 1986, compared to 35 percent in 1983 (262).

When diet and exercise are ineffective in lowering cholesterol, medication may be effective. In clinical studies, a recently approved drug (lovastatin) reduced total cholesterol by 18 to 34 percent. The drug is the first of a new class of products that inhibit the enzyme regulating the production of cholesterol (10). Other classes of available cholesterol-lowering medications include bile acid sequestering resins, nicotinic acid, and fibric acid compounds.

While an interaction of environmental and genetic factors is known to contribute to the development of atherosclerosis, an estimated 5 to 10 percent of the population is strongly genetically predisposed to the development of atherosclerosis, while another 5 to 10 percent is strongly genetically resistant. For the remaining 80 to 90 percent of individuals, both genetic and environmental factors determine who develops atherosclerosis (130).

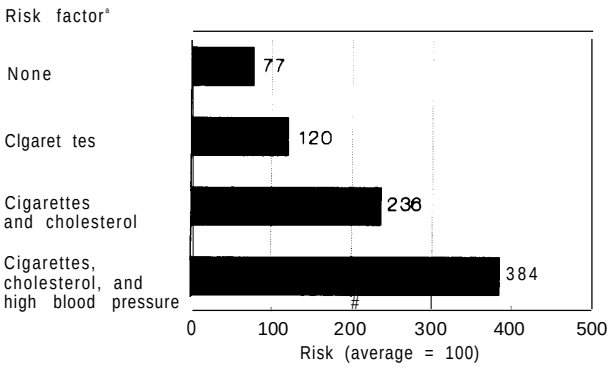
Familial hypercholesterolemia (FH) is an autosomal dominant inherited disease (i.e., the offspring of affected individuals have a so percent chance of inheriting the FH gene) and occurs with a frequency of about 1 in 500 (33). FH is caused by inherited defects in the gene encoding for the LDL receptor. These defects (at least 12 different mutations have been identified) disrupt the normal control of cholesterol metabolism (135). LDL is very elevated in those with FH and results in premature atherosclerosis (21). For those with one of the defective genes, myocardial infarction frequently ***occurs*** by age 30 to 40, and death usually occurs before age 60 (33). Treatment for FH is available, but it is necessary to institute it early to prevent the vascular complications of the disease. Examining those with a family history of early CHD is worthwhile. In fact, the American Heart Association (AHA) recommends that physicians contact first-degree relatives of all patients who have developed any clinical features of CHD under the age of about so (7) . However, this will not have a large impact on overall incidence. Among those under age 60 who suffer myocardial infarctions, only about 5 percent represent FH, and approximately 10 to 20 percent of those with one defective gene do not have myocardial infarctions until they are 80 to 90 years old, despite pronounced hypercholesterolemia from birth (33) .

Calculation of Risk Profiles.—In terms of recommended CHD screening practices, physicians have generally been advised to screen for and reduce risk factors, including tobacco use, elevated serum cholesterol, and hypertension. Routine

EKGs are not indicated as a screening test (93). The Canadian Task Force on the Periodic Health Exam states that no screening is recommended for CHD, although screening for hypertension is recommended for other reasons.

Based on the longitudinal Framingham Study, estimates of individual risks for heart disease have been calculated according to demographic, behavioral, and health characteristics. Physicians can apply the results of the Framingham Study to their evaluation of individual asymptomatic adult patients. Risk scores are calculated based on a patient's age, sex, systolic blood pressure, serum cholesterol, presence of left ventricular hypertrophy (as determined by EKG), smoking status, and presence of sugar in the urine (154). Figure 4-15 summarizes relative risks when cigarettes, high cholesterol, and high blood pressure are present. While useful, these calculations will identify many who will develop heart disease as low risk, and predict others to be at high risk who will remain disease-free. In fact, only about half the risk of CHD can be accounted for by known risk factors. For example, while both hypertension and hypercholesterolemia are recognized risk factors for CHD, two-thirds of healthy adult men ages 40-5s who are above the 80th centile for elevated cholesterol levels and blood pressure will remain well over during the subsequent 25 years (235). Therefore, although the presence of these risk factors raises one's relative risk, the absolute risk remains low.

Figure 4-15.-The Danger of Heart Attack Increases With the Number of Risk Factors Present



aThe dangers of hearl attack increase with the number **of risk factors Present.** For purposes of Illustration, this chart uses an abnormal blood pressure level of 1S0 systolic and a cholesterol level of 310 **in** a 45 year old man.

SOURCE: American Heart Association, "19S7 Heart Facts," Dallas, TX, 19S7.

DNA-based Tests for Heart Disease. -Analyses of associations between genetic variations (polymorphisms) and the occurrence of cardiovascular disease are one of the promising areas of research relating to the development of predictive tests for heart disease. Currently, disease risk assessment includes measurement of risk factors such as elevations of plasma lipids and blood pressure. Genetic tests may further refine risk assessment and can be applied early in life before signs of disease become apparent. The development of DNA predictive tests for non-infectious disorders, however, is in its infancy, and many hurdles must be overcome before they become a part of routine care. To date, several associations have been documented between specific genetic polymorphisms and disease. These initial studies will need to be replicated and prospective studies conducted before predictive tests will have clinical utility.

Genetic polymorphisms in the region of the three apolipoprotein genes (apolipoproteins A-I, A-IV, and C-III) clustered on chromosome 11 have been associated with CHD and lipoprotein abnormalities (130). Apolipoprotein A-I is the principal protein constituent of HDL-cholesterol and may promote removal of cholesterol from the arterial wall. An HDL-cholesterol level below the IOth percentile for age and sex has been observed in *60* percent of patients with CHD under age 60 (226).

A restriction fragment length polymorphism (RFLP) (see section on genetic tests for a description of RFLPs) flanking the apo A-1 gene has been used to study the association of early CHD with specific genetic variants. In one study, a 3.3 kb (kilobase) band appeared in 4.1 percent of randomly selected control subjects and 3.3 percent of *30* subjects with no angiographic evidence of CHD. In contrast, 32 percent of 88 patients with severe CHD before the age of 60 had the 3.3 kb band. The authors concluded that the relative risk of CHD associated with the presence of the 3.3-kb band is at least *10 (226).* To put this relative risk in perspective, the relative risk of CHD developing within 18 years for **a** 35-year-old male

with only high cholesterol (total cholesterol of 335) as a risk factor is estimated to be 3.9 (154).

Although significant associations between the presence of genetic variation and disease and/or lipid abnormalities have been identified in some studies, these have not been predictive of disease in all studies. This is illustrated by looking at recent work relating to the association of DNA polymorphisms within the coding sequence of the apo B gene (located on chromosome 2) with lipid abnormalities *(164)* and myocardial infarction (MI) (131). As discussed earlier, high plasma apo B levels (the protein associated with LDL) are associated with an increased prevalence of atherosclerosis. Some investigators have found specific RFLPs associated with altered plasma LDL cholesterol levels. In one study, subjects with a specific apo B gene RFLP had triglyceride, cholesterol, and apo B levels of *36, 8,* and 10 percent, respectively, higher than those without the restriction site *(164).* However, the presence of the restriction site is common. In the study group of 83 males ages 40 to 64 (enrolled in a prospective heart disease study), 60 (72 percent) had the allele (Xl) associated with higher plasma lipid levels. Furthermore, despite the observed differences in plasma lipids, the 95 percent confidence limits associated with the mean levels of cholesterol and triglycerides for each group overlapped (table 4-11), indicating that there was no statistically significant difference between the groups. Given the frequency of the Xl allele and the lack of a strong association between its presence and altered lipid levels, it does not appear as a likely disease marker.

Other investigators have studied this same polymorphism of the apo B gene and found it to be associated with MI but not with altered levels of LDL-cholesterol or apo B (131). When cases with MIs were compared with matched controls, there were significant differences between the groups with and without the XbaI restriction site. Myocardial infarction cases were more likely to have the 8.6 fragment (designated as X2 above) than controls (88 percent versus 74 percent). As these investigators did not find significant differences between the allele associated with the 8.6 fragment and LDL-cholesterol or apo B levels, they postulate that regions of the apolipoprotein B gene other than the LDL receptor binding region represent independent risk factors for MI (131).

In addition to the XbaI allele, these investigators found two other alleles that were more common among MI patients. However, none of the alleles were very predictive of MI (odds of disease, given the presence of associated markers, were less than 1.8). As the investigators point out, however, finding differences larger than these would have been surprising, given the complexity of the atherosclerotic process. A very large difference in allele frequency between cases and controls would imply that variation at one particular genetic locus is associated with the development of MI in a large fraction of cases, and this is unlikely. Further studies in which cases are selected for a clinically defined subset of persons experiencing an MI may show a stronger association. In addition, prospective studies will need to be undertaken to evaluate the predictive value of the presence of these genetic variants. It is unlikely

Table 4-il.—Serum Cholesterol and Lipid Concentrations in 83 Subjects by RFLP Genotype (Xbal Restriction Endonuclease)

	Genotype[a]		
	XlxI (n=27)	X1X2 (n=33)	x2x2 (n =23)
Cholesterol (mmol/l)[b]	5.14 (3.89-6.80)	5.19 (3.73-7.23)	4.79 (3.75-6.12)
Triglyceride (mmol/l)[b]	1.60 (0.67-3.84)	1.59 (0.46-5.60)	1.17 (0.46-2.96)
Apo B (mg/dl)[c]	85.1 +/–29.6	86.2 +/–51.4	76.7 +/–35.4

[a]Genotype X1X1 associated with one 5.0 kb fragment.
Genotype X1X2 associated with two fragments, 5.0 kb/8.6 kb.
Genotype X2X2 associated with ona 8.6 kb fragment.
bLog data analyzed; geometric mean and approximate 95% range is given.
[c]Mean plus or minus the standard deviation.

SOURCE: Office of Technology Assessment, adapted from A. Law, L.M. Powell, H. Brunt, et al., "Common DNA Polymorphism Within Coding Sequence of Apolipoprotein B Gene Associated With Altered Lipid Levels," *Lancet* 1(8493):1301-1303, June 7, 1986.

that DNA markers will replace the need for determining quantitative plasma lipoprotein or apolipoprotein levels. Instead, DNA markers will probably be used with plasma lipoprotein and apolipoprotein levels to enhance the ability to diagnose susceptibility to develop atherosclerosis (130).

Testing for Alcoholism

Alcoholism affects an estimated 10 million Americans. Among males, the prevalence of alcohol abuse or dependence is estimated to be between 8 and 10 percent and among women, between 1 and 2 percent (241). Alcoholism, the result of tolerance and physical dependence on alcohol following long-term use, was recognized as a disease in 1956 (122). The health consequences of alcoholism may include liver disease, nutritional deficiency, brain dysfunction, and an increased susceptibility to a number of other chronic disorders, such as diabetes and heart disease. Furthermore, motor vehicle accidents, industrial accidents, and family violence occur in association with alcohol use, in part as a consequence of alcohol-related sensory-motor and cognitive impairment. With such wide-ranging physiologic effects it is not surprising that alcohol is estimated to play a role in approximately 10 percent of all deaths in the United States (73).

Several methods have been employed to improve the clinician's ability to recognize alcoholism, because alcoholism is often not diagnosed during physician-patient encounters. These methods include alcoholism questionnaires, biological markers of alcohol consumption, and tests to detect the early effects of alcohol use. Future methods may include markers of vulnerability to alcoholism (e.g., genetic predisposition) and predictors of who among those who drink will develop serious health consequences of alcohol use (e.g., cirrhosis of the liver) (240).

The two most widely used questionnaires are the CAGE questionnaire (an acronym derived from four questions asked) (Box 4-B) and the Michigan Alcoholism Screening Test (MAST). The MAST questionnaire contains 24 yes-no items regarding drinking behavior and problems associated with excessive drinking. In contrast to the CAGE questionnaire, when alcoholism is detected, MAST provides some information about the severity of the problem. As screening devices, the two questionnaires are comparable in accuracy. When evaluated in outpatient settings their sensitivity ranges from 55 to 97 percent, while specificity ranges from 79 to *96* percent (57,122).

Box 4-B.—CAGE Alcoholism Questionnaire

The CAGE Questionnaire:

1, Have you ever felt you ought to Cut down on your drinking?

2. Have people Annoyed you by criticizing your drinking?

3. Have you ever felt bad or Guilt$_y$ about your drinking?

4. Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover (Eye opener)?

SOURCE: Hays, J.T. and Spickard, **W. A.,** "Alcoholism: Early **Diagnosis and Intervention,** " *J. Gen. Intern. Med., 2:* 420-427, November/December 1987,

Laboratory indicators of alcohol consumption include the blood alcohol concentration (BAC) and elevated levels of the liver enzymes gamma-glutamyl transpeptidase and glutamic-oxaloacetic transaminase. BAC may be the most under-used biochemical test in screening for alcoholism (162). The level of alcohol in the blood may indicate high alcohol consumption and provide evidence of tolerance to alcohol. However, recent consumption may not be detected, because alcohol is usually cleared from the system within 24 hours (241).

Serum levels of gamma-glutamyl transpeptidase (SGGT) are not accurate indicators of alcohol use, as there is little correlation between alcohol intake and levels of SGGT. Among known groups of alcoholics, less than one-third have elevated SGGT levels. Serum levels of glutamic-oxaloacetic transaminase (SGOT) are even less sensitive indicators, because elevation in SGOT occurs only with more severe liver damage. Taken *singly,* these tests are not accurate predictors of alcoholism, but some investigators have analyzed combinations of tests and found them useful. One group of investigators has used statistical tech-

niques (quadratic discriminate analysis) to interpret the findings of a battery of 25 commonly ordered laboratory tests (complete blood count and SMA-18) to identify alcoholics. This technique has successfully distinguished between those with biopsy-verified alcohol and nonalcoholic liver disease (252). However, this statistical technique has not been evaluated for its ability to identify those with preclinical or early-stage alcoholism.

One potentially promising method to assess alcohol consumption relies on the finding that alcohol modifies hemoglobin. Hemoglobin-carrying red blood cells live for about 120 days and can be sorted by age (110). By studying alcohol-modified hemoglobin in age-stratified red blood cells, it may be possible to assess the nature and pattern of alcohol use over the previous three-month period (256).

Early physical signs and symptoms of alcoholism may include: gastrointestinal (GI) problems (e.g., early morning vomiting, chronic diarrhea, gastritis, GI bleeding); hypertension or arrhythmias and palpitations in a patient without known heart disease; sleep disturbances; and sexual dysfunction (122).

Many studies have compared the relative effectiveness of laboratory indicators versus questionnaires as alcoholism screening tools. In almost all cases, both the CAGE and the MAST questionnaires have been shown to be superior to any of the laboratory markers used to identify alcoholics (122). Furthermore, the CAGE questionnaire has been shown to be an effective tool for detecting both alcohol-dependent and alcohol-abusing patients (34).

Recent preliminary research on another biologic-marker for alcoholism shows promise (inhibition of monoamine oxidase by ethanol and stimulation of platelet adenylate cyclase activity) (281). These markers correctly categorized 75 percent of alcoholics and 73 percent of nonalcoholic controls. Furthermore, the tests were able to detect abnormalities in alcoholics who had abstained from alcohol for as long as 23 days. If this biologic marker is a measure of the underlying basis of alcoholism (there is evidence of genetic susceptibility to alcoholism) rather than a measure of the effects of alcohol consumption, primary prevention would become possible, because susceptible individuals could be identified and counseled before they began drinking. Further research will be necessary to clarify whether this marker proves to be useful.

SELF-TESTING/HOME DIAGNOSTIC PRODUCTS

The Availability of Home Diagnostic Products

Introduction

There are now approximately 60 do-it-yourself kits available to detect a variety of conditions, ranging from pregnancy and ovulation to blood in the stool. These products have been popularized through books and journals. One such book describes how to correctly obtain a urine and blood sample at home and how to conduct and interpret more than 160 screening tests, including tests for diabetes, hypertension, sickle cell disease/trait, gonorrhea, and alcoholism. The authors do not suggest home medical testing as an alternative to physician-ordered testing but rather as an adjunct to it (234).

Self-administered tests may be valuable in identifying signs of latent disease. Asymptomatic individuals can test themselves for hypertension and colon and breast cancer using over-the-counter (OTC) testing products. Prompt medical attention at an early stage of disease may prevent or ameliorate the course of the disease. Those with symptoms of illness may screen themselves for strep throat infections, urinary tract infections, and some kinds of sexually transmitted disease.

The largest market for home testing has been in the area of therapeutic monitoring. Diabetics

monitor their insulin control through urine and blood glucose tests, and some patients with heart disease monitor drug levels. Market forecasters predict substantial growth in the home testing market (table 4-12). This may be attributed in part to the aging of the American public. By the year 2000, an estimated 13 percent of the population will be aged 65 or older (292). Given the frequency of those 65 or older who have at least one chronic condition, these products, especially those used to monitor therapeutic control, may be widely used.

The number of home testing products has increased largely as a result of technological advances that have simplified tests. For example, accurate, easy-to-use, enzyme-linked immunoassay have shifted testing away from clinical labs to physicians' offices. Many of the tests now available to the consumer are the same as those used by physicians (95).

While some argue that home testing may facilitate early treatment and therefore better health outcomes, others are concerned that consumers will misuse or misinterpret test results and delay or not seek needed medical care. There is also concern that the widespread use of tests with inherent false positive results may lead to unnecessary physician visits and expensive follow-up evaluations. While physicians believe that patient self-diagnosis is on the ***rise,*** their opinion on self testing is divided. Physicians favor urine glucose/ketone, blood glucose, and occult fecal blood testing, but are generally opposed to self-diagnosis of urinary tract infections, sexually-transmitted diseases and breast cancer (via thermography) (23).

An extension of home testing is the development of self-service laboratories, where individuals may order a variety of clinical tests without the participation of a physician. For example, a California-based, private laboratory, offers 36 different tests directly to the public. Included are tests for early detection of disease/infection (e.g., HIV antibody testing), indicators of exposure to toxic industrial substances, nutritional and allergy profiles, drug testing, and standard blood and urine workups (e.g., glucose, cholesterol, and triglyceride levels). The laboratory is run by a physician who refers clients to a physician if warranted by the test results (28).

Some laboratories have sold kits in which the consumer collects a specimen (usually blood by the fingerstick method) and sends it to the lab for analysis. Even though the test kit that is sold to the consumer contains FDA-approved components (e.g., lances, swabs, blood collection tubes) manufacturers of such kits are required to notify the FDA prior to selling the kits. Manufacturers of such kits have withdrawn them from the market and have applied to the FDA for their ap-

Table 4-12.—Summary of Market Research Reports on Home Testing Products

Market researcher/product	Year	Market value (millions)	Year	Market value (millions)
Find-SVP				
Entire market	**1986**	**$668**	***1995***	**$2,200**
Fecal occult kits	**1986**	**$33**	***1995***	**$ 136**
Breast cancer screening kit	**1986**	**$ 1**	***1995***	**$ 7**
Blood pressure monitoring	**1983**	**$69**	***1986***	**$ 100**
Packaged Facts				
Entire market	**1987**	**$515**	1992	**$1,400**
Biomedical Business International				
Entire market	**1986**	**$432**	**1990**	**$1,200**
Blood glucose test	**1986**	**$205**	**1990**	**$ 445**
Blood pressure monitoring	**1986**	**$112**	**1990**	**$ 211**
Urine glucose	**1986**	**$ 19**	**1990**	**$ 15**
Fecal occult kits	**1986**	**$ 3**	**1990**	**$ 9**
Business Communications Co.				
Entire market,			1990	$2,500
Frost and Sullivan				
Entire market	**1984**	**$348**	**1989**	$ 736

SOURCE" Office of Technology Assessment, 1988

proval. As of early 1988, such test kits have not been approved by the FDA.

The Market for Home Tests

Estimates of the market value for home diagnostic products by 1989-1995 vary from $736 million to $2.5 billion (see table 4-12). The projections vary in part because of differences in what is considered a home test. Some estimates include the projected sales of thermometers and "home care products" such as condoms. Earlier projections that sales of self-testing products would exceed $1 billion by the mid-1980s have not been realized. Although actively promoted, home pregnancy tests are used by only 7 percent of women of child-bearing age, and only 9 percent of all households have blood pressure kits (23). An estimated 20 percent of insulin-dependent diabetics use blood glucose kits (22). Among the barriers to market penetration are that many consumers do not want to know about their disease; rely on their physicians for tests; are unwilling to pay for OTC testing products; are intimidated by testing technology; and lack knowledge of health problems that would enable them to consider self-testing (23). Self-testing products are used by the better-educated and higher-income consumer (77). Some of these barriers may be overcome by aggressive marketing. Retail outlets for tests include drug stores, physician offices, HMOS, nursing homes, health clubs, and businesses.

Accuracy and Reliability of Home Tests

The major concern regarding home diagnostic tests is that false negative test results may lead to a delay in treatment and that false positive test results may lead to unnecessary follow-up testing and emotional stress. Erroneous results may occur through test misuse or because of inherent limitations of the tests themselves.

There are two reporting systems established to identify problems associated with medical devices, including home diagnostics. First, health care practitioners can, on a voluntary basis, report problems to The Medical Device & Laboratory Product Problem Reporting Program, which is administered by the United States Pharmacopoeia Convention, Inc. and funded by the FDA. The program is interested in receiving information about design defects, device malfunctions, improper packaging, questionable sterility, and inadequate labeling or instructions **(18).**

A second reporting system, the FDA's Medical Device Reporting (MDR) system, includes reports of device-related injuries, deaths, and device malfunctions that could lead to injury or death. Manufacturers are required to report such experiences to the MDR system. Approximately 1,600 reports of injuries and malfunctions have been logged with the MDR system for home glucose screens alone (from December, 1984 to June, 1987), mostly due to improper use (63). The FDA is evaluating the source of the problems, and if the patient education process is implicated, manufacturers may be required to provide clearer device labeling and less complex training literature *(64).*

The FDA, in recognition of potential problems associated with home test kits, is in the process of issuing guidelines to the manufacturers of such products. In the proposed guidelines, FDA suggests that manufacturers demonstrate that home test kits show "probable health benefits" and that they can be operated easily. Firms are also advised to prove that there are benefits of performing the test at home instead of having the test performed by health care professionals. Manufacturers will also be required to document the impact of a false positive or false negative test result to the user or to society (e.g., delay in seeking medical care). Companies will be required to submit data showing that home tests "perform as well as their professional-use in vitro diagnostic equivalents . . . [and] should be designed . . . to ensure that performance will not be appreciably affected by anticipated variation in user technique" **(63).** Most home test kits will be approved by the relatively short premarket notification process. The currently available FDA-approved HIV tests are licensed for blood bank and lab usage, but not for home or in-office use.

Conclusion

Currently, there are few home diagnostic tests that would lead an insurer to expand their use of diagnostic testing. However, with improvements

in technology, a wider variety of home diagnostics may be available. These include tests for disease susceptibility such as HIV infection (see AIDS section), and colon cancer (improved occult fecal blood tests). Genetic probe tests suitable for home use will not be available in the near future (see genetic testing section). However, if such tests are available and they are widely used, insurers may consider expanding their testing efforts to avoid adverse selection.