

Prenatal Screening at the Wisconsin State Laboratory of Hygiene

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INTRODUCTION

Entry of public health laboratories into the medical arena of prenatal health evolved from their traditional role in communicable disease control, including sexually transmitted diseases. At the turn of the 20th century, syphilis was a major health problem, resulting in high morbidity and mortality rates in Wisconsin. Furthermore, four centuries before 1900, syphilis had been linked to abnormalities of the newborn.¹ Prevention of congenital syphilis as a public health activity motivated Wisconsin and other states to enact premarital laws and to conduct other control efforts. In 1912, the Mendota State Hospital opened a laboratory using the recently developed Wassermann test to investigate institutionalized patients. This laboratory was transferred to the University of Wisconsin Psychiatric Institute in 1925, where new testing procedures were developed. In 1951, this activity was assumed by the Wisconsin State Laboratory of Hygiene (WSLH). Around this time, other congenital infections were recognized, which stimulated the WSLH to develop a testing program for congenital infections.

DEVELOPMENT OF THE PRENATAL SCREEN

Although toxoplasmosis in the newborn was first reported in 1923,² it was the development of the Sabin dye test in 1948 that enabled laboratories to diagnose suspected cases of toxoplasmosis.³ When epidemiological studies identified undercooked meat⁴ and cat feces⁵

as sources of infection during pregnancy, these provided an additional impetus for testing pregnant women. In 1941, Gregg identified rubella as another infectious teratogen,⁶ and in 1962 rubella virus was first isolated in tissue culture.⁷ The WSLH introduced rubella virus isolation that same year and, in 1964, the rubella indirect neutralization test was offered for serologic diagnosis. Another viral agent, cytomegalovirus, was recognized in 1957 as a potential teratogen.⁸

During the 1950s and 1960s, the WSLH devoted increasing resources to develop and improve individual serologic assays. Recognizing the need for a unified approach to benefit physician and patient interests, in 1973 the Immunology Section introduced the Prenatal Screen Panel, consisting of assays for ABO group, Rh type, atypical blood factor antibody, *Toxoplasma gondii*, *Treponema pallidum*, and rubella immune status. The panel assays were performed on a single blood specimen and provided rapid turnaround time and coverage of the most common manageable forms of congenital disease. Through the ensuing years, many modifications were made to the panel as newer procedures and epidemiologic information became available. In addition, the WSLH started a serum bank, the first of its kind in prenatal testing, in which sera were stored frozen for a period of 1 year. Retention of specimens permitted comparison of antibody titers when second specimens were taken later in pregnancy. Cytomegalovirus and herpesvirus serology could also be requested on paired sera when viral infections were suspected prior to delivery or postpartum.

Another unique feature of the Prenatal Screen program was the distribution of *Prenatal Screen Notes*, a quarterly newsletter for physicians and laboratorians. The WSLH also conducted laboratory workshops on prenatal testing, often with assistance from scientists at the Centers for Disease Control and Prevention.

NEURAL TUBE SCREENING

On July 1, 1977, after 1 year of research in the Immunology Section, a test for alpha1-fetoprotein (AFP) was

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Table 1. Outcome of Pregnancies with Initial Abnormal Maternal Serum AFP (MSAFP) Levels, 1977-1985

Total number of pregnancies screened	77,457
Number of pregnancies with raised MSAFP on initial screening	1,899 (2.5%)
Errors in estimated gestational age, normal outcome	445 (23.4% of 1,899)
Number of abnormal outcomes or multiple gestations	619 (32.6%)
Multiple gestations	208
Prematurity	140
Spontaneous or missed abortions, fetal demise, stillbirth	110
Neural tube defects detected	55
Other major defects (renal dysgenesis, Turner syndrome, etc.)	35
Abdominal wall, cord, or alimentary tract defect	21
Placental defect	25
Low birth weight	25

Table 2. 1992—Prenatal Panels

Entry Prenatal Panel	Second Trimester Prenatal Panel
ABO Group	MSAFP* and Adjusted MoM†
Rh Type	BhCG‡ and Adjusted MoM
Atypical Blood Factor Antibody	uE3§ and MoM
Venereal Disease Research Laboratory	Down Syndrome Risk Calculation
Neural Tube Defect Risk	Rubella Immune Status
	Calculation
Toxoplasma IgG and IgM	
Hepatitis B Surface Antigen	

*MSAFP. Maternal serum alpha-fetoprotein
 † Multiple of the median
 ‡ BhCG. Beta human chorionic gonadotrophin
 §uE3. Unconjugated estriol

added to the Prenatal Screen, with support from the Wisconsin Title V Maternal and Child Health Plan. Wisconsin became the first state to offer this service. Elevated maternal AFP during the 15th to 20th week of pregnancy, followed by amniocentesis, had been shown to detect reliably open neural tube malformations (e.g., anencephaly, meningomyelocele, spina bifida).⁹ During the early years of this program, investigations at WSLH resulted in development of a sensitive in-house quantitative radioimmunoassay (RIA) test for the screening blood test and the confirmatory testing of amniotic fluids.¹⁰ As commercial RIA and other assays became available, they were evaluated and adapted, replacing the in-house procedure. The development of increasingly accurate test procedures opened a door for several

important studies to determine if AFP could detect other conditions, including congenital nephrosis, intrauterine growth retardation, twin pregnancies, and fetal death. The ongoing generation of gestational age-specific normal values was crucial to the success of this program. As shown in Table 1, almost one-fourth of initially elevated AFP findings were attributable to errors in dating gestational age. Later, improvements in ultrasonography markedly decreased this source of error. Table 1 also shows that AFP screening detected many previously unknown multiple pregnancies, and that elevated AFP is associated with a number of adverse pregnancy outcomes. Fifty-five neural tube defects were detected as were many other malformations.

In part, because of the highly charged political nature of prenatal diagnosis, the Food and Drug Administration (FDA) proposed in 1980 to require federal licenses for both laboratories and physicians to perform AFP screening on pregnant women. A position statement against licensure, drafted by the WSLH and supported by many Wisconsin physicians, was presented at FDA hearings in January 1981. Subsequently, FDA dropped the licensure consideration and later approved AFP test kits specifically for use in prenatal diagnosis. The laboratory manufacturing industry was thereby encouraged to develop better systems, and the more difficult RIA test was replaced by commercial enzyme immunoassay (EIA) test kits. In March 1986, the WSLH received its 100,000th Prenatal Screen specimen.

MULTI-PANEL PROGRAMS

Scientific advances led to major changes in the Prenatal Screen Panel in the 1990s. In 1991, hepatitis B surface antigen testing was implemented as a new Wisconsin Division of Public Health program for screening pregnant women. Through the decade, several changes were made in testing and reporting of AFP results. Since maternal serum AFP (MSAFP) values are closely correlated with gestational age, rising to a high level between 28 and 32 weeks of gestation and then decreasing until delivery, laboratories that conduct MSAFP testing must determine median levels for each week of gestation based on large numbers of patient results. Individual results are reported as multiples of the median (MoM), and specific MoMs are considered low or high depending on gestational age. In 1991, the WSLH reported AFP values in ng/ml and MoMs, and a position statement on interpretation of results was adopted in cooperation with the Wisconsin Association of Perinatal Care.

In 1992, a major change was implemented, as the

WSLH began offering 2 prenatal panels. The Entry Prenatal Panel, designed for the patient's initial visit, did not contain the Maternal serum alpha-fetoprotein MSAFP test (Table 2). The Second Trimester Prenatal Panel was recommended to be performed between 14 and 20 weeks gestation. This new panel was designed to improve neural tube defect risk calculation, but also to permit risk calculation for Down Syndrome. The 3 serum markers in the Second Trimester Prenatal Panel (alpha-fetoprotein [AFP], beta human chorionic gonadotrophin [BhCG], unconjugated estriol [uE3]) had been found in epidemiological studies to be predictive of fetal Trisomy 21 (Down Syndrome). Prior to the availability of prenatal serum testing, risk for Trisomy 21 was based primarily on age, with the risk increasing markedly for older women. Age 35 was generally considered the age at which a woman might consider amniocentesis for AFP assay. Since about 90% of all infants are born to younger mothers, and since 70% of trisomic infants are born to this population, despite the lower risk, younger women could therefore benefit from a triple screen risk assessment.

THE TRIPLE SCREEN AND BEYOND

The triple screen introduced a level of complexity to laboratory testing not previously experienced. First, each component was measured by highly sensitive techniques—AFP and uE3 in ng/ml and BhCG in IU/ml. Each value was then expressed as an MoM based on the week of gestation. In addition, extensive research had determined that the risk calculation was influenced by other maternal factors. African-American women tend to have higher MSAFP values; type 1 diabetics have lower MSAFP, BhCG, and uE3 levels. Women who are heavier tend to have lower values. Thus, for accurate risk assessment, a careful history is essential. To accomplish the highest quality testing program possible, the Immunology Section recognized the requirement for complete and accurate patient information. Instructional pamphlets were provided to physicians describing the laboratory procedures, prenatal screen requirements, and the submission of specimen procedure. A detailed requisition form was provided to obtain accurate information on patient age, race, gestational age, number of fetuses, obstetrical history, height and weight, and presence/absence of diabetes.

To estimate the risk of carrying a fetus with Down Syndrome, a multivariate model allows the values of the 3 analytes, corrected by the supplementary personal factors, to be combined with maternal age to determine

the likelihood of trisomy. Computer software programs became available to carry out the complex statistical analysis. Thus, unlike other types of laboratory reporting where tests are expressed as presence or absence of an organism (microbiology) or concentration with normal limits (clinical chemistry), second trimester prenatal testing introduced risk calculation using multiple analytes.

Another critical aspect of this prenatal program is patient follow-up. Wisconsin physicians utilizing this service were very conscientious in providing pregnancy outcomes, so that results could be correlated with the risk assessment. Follow-up data were provided in 94% of pregnancies considered to be at risk for Down Syndrome during the initial years of the Second Trimester Panel. In 1996, the program began providing risk assessment for Trisomy 18. Prenatal Screen users were provided a WSLH flowchart with the recommended procedure to follow in case of an abnormal triple screen result. The flowchart gave guidance for evaluating increased risk reports for Trisomy 18, Down Syndrome, and neural tube defects. The Second Trimester Prenatal Panel was a very successful program that served primary care physicians and obstetricians throughout the state. In the late 1990s, manufacturers of laboratory equipment and reagents marketed products that enabled the private sector laboratories to initiate triple screen testing. Increased availability in hospital and independent laboratories, plus patent-related issues, motivated the WSLH to discontinue the Second Trimester Panel in 2002.

There were also several changes in the Entry Prenatal Panel in the late 1990s. In 1995, 4 options were made available in response to requests from many physicians—basic panel, basic plus HIV-1/2, basic plus toxoplasmosis, and basic plus HIV-1/2 and toxoplasmosis. At that time, the Basic Entry Panel consisted of ABO, Rh, blood factor antibody, rubella immune status, VDRL, and hepatitis B surface antigen. In 1998, options for hemoglobin electrophoresis and varicella immune status were added. Currently, 6 panels are offered, including the 4 listed above and 2 specially designed for the Division of Public Health.

PRENATAL CYTOGENETICS AT THE WSLH

In the early 1960s, a cytogenetics laboratory activity was started at the WSLH in cooperation with pediatricians and clinical geneticists at the University of Wisconsin (UW) Medical School. The initial interest was in detecting chromosomal abnormalities in children with mental retardation and multiple congenital

malformations. These early pioneering studies were productive in describing previously unrecognized syndromes.^{11,12} In the mid-1960s, support from the Wisconsin Bureau of Maternal and Child Health helped to support the expansion of these investigations. Large-scale studies were carried out to determine the role of chromosomal abnormalities in spontaneous human abortion¹³ and in patients in Wisconsin institutions for the handicapped.¹⁴

Chromosome analysis is a process that requires the evaluation of the 46 chromosomes in well-prepared metaphase plates. Preparation techniques are required to obtain dividing cells from a variety of sources to enable examination of a sufficient number of metaphase spreads in order to make an accurate diagnosis. In the early 1960s, preparation techniques were crude and did not permit distinguishing chromosomes in certain groups from one another. Autoradiographic methods offered little advantage. In the early 1970s, methods were developed to create distinctive bands in the individual chromosomes, thus enabling more precise identification.¹⁵

Following reports that it was possible to grow fetal cells from amniotic fluid,¹⁶ the cytogenetics laboratory began offering this testing service in 1970 to Wisconsin physicians in cases of high-risk pregnancies, which at that time related mostly to mothers over age 35. When the triple screen was introduced in 1992, which identified at-risk pregnancies in women younger than 35, amniotic fluid submissions greatly increased. By 1999, more than 1200 specimens were being submitted annually. Thus, a laboratory technique that was originally used to identify the cause of abnormal development in affected children and families assumed a public health role for screening healthy pregnant women not considered at increased risk for birth defects.

In 1985, the WSLH cytogenetics laboratory developed techniques to culture cells from chorionic villus biopsies, starting at 11 weeks of gestation, in cooperation with clinicians from the Department of Obstetrics and Gynecology at the UW Medical School. By successfully culturing these cells, it became possible to identify chromosomal abnormalities in the first trimester of pregnancy. Soon other Wisconsin physicians began using this method of prenatal diagnosis in pregnancies considered to be at high risk due to maternal age or family history. About 250 such tests are performed annually in the cytogenetics laboratory. In addition, transabdominal chorionic villus biopsies, which can yield results in 24 hours, may be used in mid-trimester pregnancies in which an immediate diagnosis is required.

TECHNOLOGICAL ADVANCES IN CYTOGENETICS

The 1990s witnessed an explosion in the application of new genetic technology in medicine. These advances resulted from progress in the human genome project, increased funding for genetic research in universities, a proliferation of venture capital-sponsored biotechnology companies, and the promise of gene therapy. At the same time, many questions and concerns were being expressed on the medical, scientific, ethical, legal, and social issues raised by the development and use of genetic tests. A federal Task Force on Genetic Testing in the mid-1990s expressed concerns about the ways in which tests are introduced into clinical practice, and the adequacy and regulation of laboratory quality assurance. Subsequently, the Secretary of Health and Human Services formed the Secretary's Advisory Committee on Genetic Testing (SACGT) in 1999. The SACGT recommended in September 2001 that the FDA develop a pre-market review process for genetic tests.¹⁷

In the 1990s, fluorescent DNA probes that hybridize with specific chromosomes and regions of chromosomes became available from biotechnology manufacturing companies. At first, centromere probes enabled rapid enumeration of chromosomes, even in non-dividing cells. Shortly thereafter, whole chromosome painting probes, as well as specific locus probes utilizing fluorescent dyes were introduced. Fluorescent in situ hybridization (FISH) technology not only facilitated chromosome identification, but it enabled detection of microdeletions not visible in even the most elongated metaphase spreads. Deletion detection has been applied successfully in Prader-Willi, DiGeorge, and many other genetic syndromes. FISH probes have also confirmed suspected cytogenetic rearrangements, enabling family studies to detect carriers at risk for birth defects.

Recent advances in FISH technology now include telomere probes, which can detect tiny rearrangements at the tips of chromosomes, which are not detectable by other methods. Demonstrating such a cytogenetic aberration as the cause of an atypical malformation syndrome can obviate further diagnostic testing. Use of FISH to detect cryptic chromosomal aberrations can aid in family planning. Furthermore, detecting a chromosome rearrangement, such as a translocation between chromosomes 14 and 21, (Figure 1), enables the use of prenatal cytogenetic diagnosis to assure that the chromosome constitution will not lead to abnormal development, which is the case in more than 85% of preg-

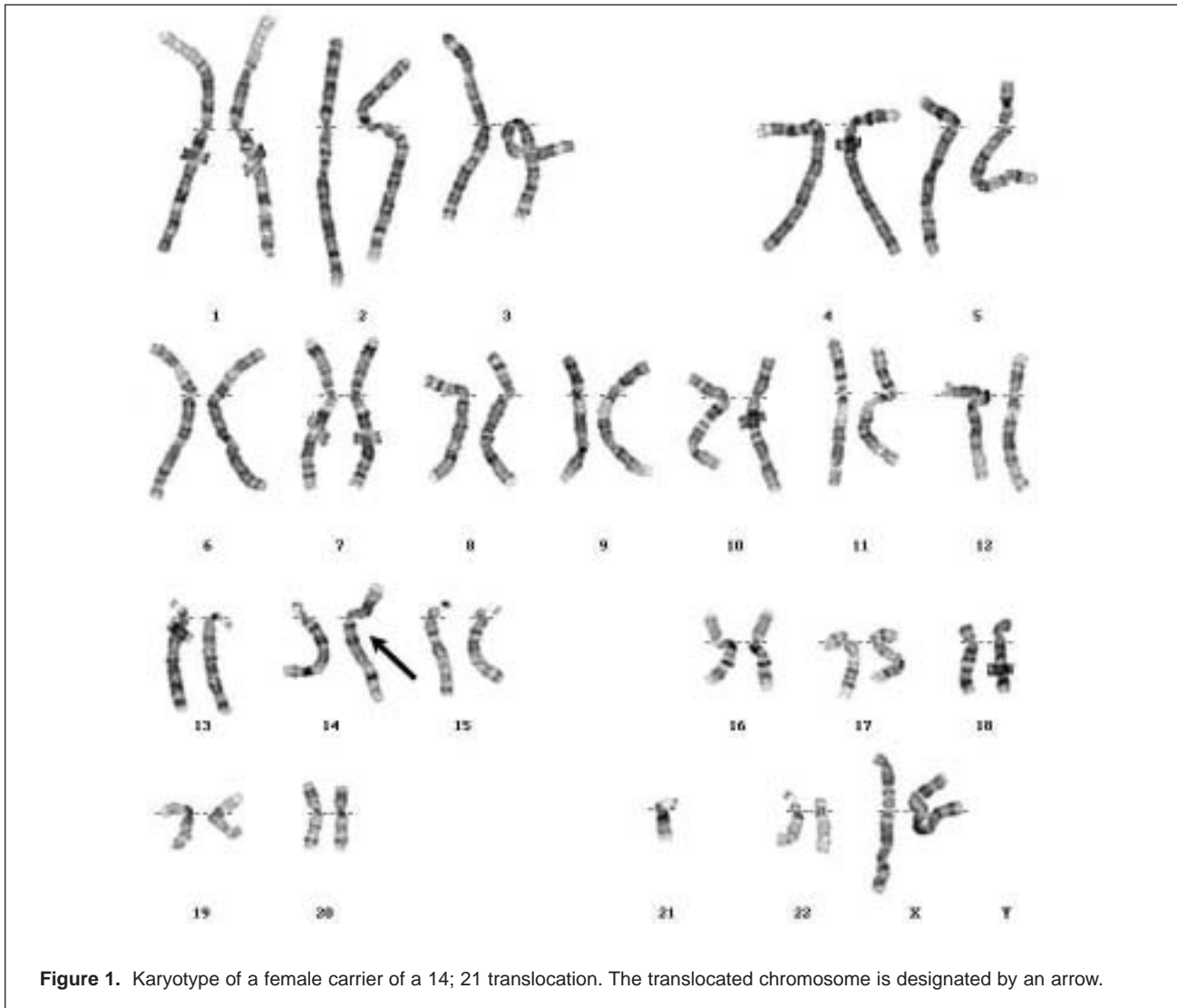


Figure 1. Karyotype of a female carrier of a 14; 21 translocation. The translocated chromosome is designated by an arrow.

nancies when the mother is a carrier and more than 95% when the father carries the translocation.¹⁸

The traditional method of chromosome analysis involves photographing 10 to 15 evenly spread, well-banded dividing cells, and manually cutting out individual chromosomes to prepare 2 or more karyotypes. Microscopic analysis and karyotype evaluation often requires several hours of cytogeneticist time. Additional banding procedures are sometimes required. Computerized imaging systems were developed in the 1990s that provide software for digitizing the microscopic images of the chromosomes. With guidance by the cytogenetics technologist, images can be rapidly arranged in a karyotype. This technology was instituted in 1997 at the WSLH, and upgrades have been added. The system also permits long-term storage of dividing cell and karyotype images, and provides the

ability to enlarge and enhance individual chromosomes for increased resolution. The laboratory can print hard copies for the physician and, when desired, for the patient.

CONCLUSION

In the development of both the Prenatal Panels and Prenatal Cytogenetics programs at the WSLH, the laboratory has been a pioneer in providing state-of-the-art screening and diagnostic services to Wisconsin physicians. These activities have been made possible by research and collaboration with clinicians, geneticists, laboratory reagent and equipment manufacturers, genetic counselors, and professional colleagues at the University of Wisconsin and throughout the country. In all these endeavors, a goal has been to assure that the citizenry of Wisconsin has access to these advanced lab-

oratory techniques by providing services directly and also by transferring technology to other laboratories in the state. The pace of development in the fields of reproductive technology, genomics, and gene therapy promises to accelerate in the years ahead. Medical genetics promises to extend to all fields of medicine at all stages of the life cycle—prenatal, perinatal, childhood, and adulthood. The expansion of genetic medicine will impact public health policy as well as delivery of services.^{19,20} With its state-of-the-art prenatal and newborn screening programs, Wisconsin is in an excellent position to meet the challenges of the genetic revolution in the 21st century.

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