
Maternal Serum Screening; Approved Standard

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Abstract

NCCLS document I/LA25-A—*Maternal Serum Screening; Approved Standard* is written for clinical laboratorians who participate in prenatal screening for open neural tube defects and Down syndrome involving AFP, hCG, uE3, DIA, and/or PAPP-A measurements, as well as for clinicians and manufacturers who have a direct interest in the tests. The standard is intended to present necessary considerations: preanalytical, analytical, and postanalytical; and to ensure the reliability of the tests, including the risk calculation, the outcome evaluation, and the accuracy of the information management. If properly applied, the four biochemical determinations (or five, if performing integrated testing with PAPP-A from the first trimester), and the risk calculations can contribute constructively to the field of prenatal screening and to the welfare of pregnant women and the fetus.

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Foreword

The goal of this document is to update information on second-trimester maternal serum screening (MSS) for open neural tube defects (NTD), which occur at an incidence of 1 to 2 in 1000 live births, and Down syndrome (DS), which occurs at an incidence of 1.6 in 1000 live births. Even with the advent of first-trimester screening markers for DS, second-trimester testing is important due to the following:

- The need for second trimester AFP screening for NTD.
- A significant proportion of pregnant women do not seek prenatal care until the second trimester, thus the need for accurate determinations of biochemical markers of second-trimester MSS.
- First-trimester screening relies on, in addition to the biochemical markers choriogonadotropin free β hCG and pregnancy-associated plasma protein-A (PAPP-A), a nuchal translucency measurement which requires qualified ultrasonographers or physicians experienced in ultrasound which are not always available.
- Without a nuchal translucency measurement, screening using the two first-trimester biochemical markers alone has only about a 60% detection rate for a 5% false positive rate, and is, therefore, not recommended as a screening method. However, a screening protocol using the PAPP-A measurement from the first trimester together with the triple test markers (AFP, uE3, hCG) or the quadruple test markers (AFP, uE3, hCG, DIA) in the second trimester, offers an effective method of screening (the serum integrated test). This improves the performance of second-trimester screening.

This document updates, extends, and replaces NCCLS document I/LA17-A—*Assessing the Quality of Systems for Alpha-Fetoprotein (AFP) Assays Used in Prenatal Screening and Diagnosis of Neural Tube Defects; Approved Guideline*. This document offers guidance that may be used by manufacturers and clinical laboratories that provide prenatal diagnostic services. This document addresses the standards that should be maintained by manufacturers, laboratories, and clinicians when providing screening services used to evaluate pregnancies and risks of fetal disease. At this time, the principles of serum screening remain similar regardless of which assay(s) is/are used as part of the evaluative service. The standard addresses the steps required to provide reliable screening and reporting using examples of serum markers currently in common use (AFP, hCG, uE3, DIA). It is recognized that the list of assays and methods of pregnancy screening will continue to change. Outcome evaluation, information management, and calculation of risk are also emphasized in this standard. Screening for Down syndrome (T21) also includes the incidental detection of Edwards syndrome (T18).

Unlike diagnostic testing which is designed to make the diagnosis of a specific disorder, screening is intended to identify individuals with a sufficiently high risk of that disorder to benefit from further diagnostic testing. NCCLS document I/LA17-A—*Assessing the Quality of Systems for Alpha-Fetoprotein (AFP) Assays Used in Prenatal Screening and Diagnosis of Open Neural Tube Defects; Approved Guideline*, published in 1997, was the first NCCLS document to outline the use of specific testing during pregnancy to assess the welfare of the pregnant woman. The part of this document not related to maternal serum screening, but to AFAFP for detection of NTD, is given as an Appendix.

To maintain historical continuity in the field, the foreword from that document (with up-to-date corrections) is quoted below:

“The aim of this document is to increase the reliability of alpha-fetoprotein (AFP) laboratory testing during the second trimester of pregnancy. Although this document primarily addresses the clinical laboratorians who perform AFP measurements for birth defect assessment, it should also be of value to clinicians and to manufacturers of reagents used in these tests. If properly applied, the measurement of AFP concentration in both maternal serum and amniotic fluid can contribute

constructively to the field of prenatal diagnosis and to the welfare of pregnant women and the fetus.

The value of AFP in prenatal diagnosis was first suggested in 1972 with a report by Brock and Sutcliffe, who documented that amniotic fluid alpha-fetoprotein (AFAFP) levels were increased in the presence of fetal open neural tube defects (NTD). The two major types of NTD are anencephaly and open spina bifida. Following confirmation of this discovery, measurement of AFAFP levels rapidly became part of second-trimester prenatal diagnosis. At that time, such testing was reserved for women who had already borne a child with a neural tube defect because these women are at increased risk for having another similarly affected child. AFP analyses were soon performed on amniotic fluid samples being processed primarily for other diagnostic purposes (e.g., chromosomal analysis).

Although AFAFP measurements proved to be diagnostically useful, occasional false-positive results led to the need for continuing reappraisal, including repeat amniocentesis in some cases. Fetal blood contamination of the amniotic fluid sample was found to be the most frequent explanation of such false-positive results. Other fetal lesions (e.g., open ventral wall defects and the Finnish type of congenital nephrosis) were also found to be associated with elevated AFAFP concentrations, thus diminishing the diagnostic specificity of the test but increasing the range of identifiable fetal problems. Requirements for sample collection and handling, as well as methodology, were such that existing prenatal diagnostic facilities in the United States were able to develop AFAFP testing capabilities with relative ease.

Leek, Ruoss, Kitau, et al, and Brock, Bolton, and Monaghan independently demonstrated increased maternal serum AFP (MSAFP) levels in the presence of fetal anencephaly. In 1974, Wald, Brock, and Bonnar, and Brock, Bolton, and Scrimgeour presented data showing that MSAFP levels were also increased when the fetus was affected by open spina bifida. Strengths and limitations of this screening procedure were addressed by numerous centers during the ensuing years, and it became clear that MSAFP measurement could be applied usefully as a routine prenatal screening test.

Two major United Kingdom collaborative studies, one in 1977 addressing MSAFP screening, and the other in 1979 involving AFAFP analysis, established the overall reliability of both analytical processes and formed a general basis for estimating detection rates and false-positive rates when applied to the United States pregnancy population. The application of AFP measurements to the antenatal diagnosis of NTD was comprehensively evaluated and reported at three international conferences (1977, 1978, and 1980) in Scarborough, Maine. As a prelude to the introduction of MSAFP screening in this country, Haddow and Macri, and Wald and Cuckle also reviewed the current state of knowledge concerning practical applications of MSAFP testing at that time. A National Conference on MSAFP was held in July of 1980 in Washington, D.C., to discuss scientific, medical, ethical, legal, and economic issues in the prenatal screening and diagnosis on NTD. Potentially, all pregnant women might be offered this blood test as a way of screening and diagnosing fetal NTD, with diagnostic ultrasound and AFAFP testing to be offered to women with positive screening results.

Although this disorder is one of the most common serious congenital malformations, people afflicted with open spina bifida may lead productive and satisfying lives. More than 2000 pregnancies are affected with this condition each year in the United States (another 2000 are affected with anencephaly). More than 95% of all open neural tube defects occur among pregnant women with no known risk factors (e.g., a neural tube defect in a close relative or previous pregnancy). All of the infants born with anencephaly will die at, or shortly after, birth. Each year, about 1400 of the infants born with open spina bifida will survive for at least five years, the majority with a significant handicap. Identifying open spina bifida prenatally allows the family to

choose between terminating and continuing the pregnancy. When the latter choice is made, the family and physician can prepare for the birth of an affected child. This advance notice permits these women to have their babies in hospitals that can offer surgical, medical, and other care needed to minimize the infant's disability. In addition, elevated MSAFP levels may help to identify pregnancies at higher risk for perinatal complications and also about 50% of twin or higher multiple-birth pregnancies.

When implementing an MSAFP screening program, it is necessary at the outset to educate physicians, nurses, laboratory staff, and the patient population of childbearing age. The differing prevalence of NTD for different populations and geographic regions is an important consideration, not only for the educational process, but also for decision making in clinical laboratories that are contemplating performing AFP analysis. AFP testing is no different from any other clinical laboratory procedure whose goal is to improve the quality of patient care. The modern laboratory needs not only to provide accurate assays, but also to aid in the appropriate interpretation of its results. By presenting guidelines aimed at assuring the quality of AFP laboratory testing, this document represents one step toward achieving that goal.”

In 1984, it was reported that, on average, MSAFP levels are about 25% lower in DS-affected pregnancies than in unaffected pregnancies.¹ Subsequently, it was shown that fetal DS affects several other MSMs. In 1987, human choriongonadotropin (hCG) was found to be elevated in maternal serum from DS pregnancies.² In 1988, maternal serum unconjugated estriol (uE3) was shown to be significantly reduced in DS pregnancies.³ In the same year, the triple test was described in which AFP, uE3, and hCG are used together with maternal age as a single screening test.⁴ In 1995, the same markers were used to identify Trisomy 18 (T18).⁵ In 1996, Wald⁶ proposed Dimeric Inhibin-A (DIA) as a fourth screening marker and in 1999,⁷ he proposed integrated screening using first- *and* second-trimester tests together to obtain a single screening result.

The capacity to measure substances derived from the embryo or fetus, through maternal blood collection, permitted the expansion of medical care to the prenatal, or antenatal, period of development. The use of MSS has expanded rapidly since the early 1970s when the first major works were published confirming that fetal alpha-fetoprotein could be measured in the maternal blood and that high levels were associated with NTD. Since then, additional markers have been identified, which permits the risk assessment of DS as well as the detection of other fetal abnormalities such as T18.

Prenatal screening services are best designed on a program basis that addresses all issues associated with prenatal care. These include a description of the population being screened, the conditions being screened, and regular audits of the screening program. In addition, attention must be given to the education of health professionals, the provision of appropriate information for women considering screening, and education of the public at large.

Key Words

Alpha-fetoprotein, amniocentesis, amniotic fluid, β hCG, chromosomal abnormalities, dimeric inhibin A, Down syndrome, human choriongonadotropin, inhibin-A, maternal serum screening, open neural tube defects, pregnancy-associated protein A, prenatal diagnosis, Trisomy 18, unconjugated estriol

Maternal Serum Screening; Approved Standard

1 Scope

This standard specifies requirements and recommendations for maternal serum screening to ensure that screening methods and quality control procedures are carried out to a high standard. It is the intent of this document to strike a balance between being sufficiently specific to be clear but not too prescriptive, allowing laboratory directors to use their professional judgment in setting policy.

The intended users of this standard are manufacturers, diagnostic laboratories, regulatory agencies, and public health authorities involved in providing or regulating prenatal screening services used to evaluate pregnancies and risks of fetal disease.

2 Introduction

Prenatal screening for serious fetal abnormalities has made significant advances over the last 25 years when maternal serum alpha-fetoprotein started to be used as a screening test for open neural tube defects. Additional maternal serum measurements have been shown to be useful, for example, in screening for Down syndrome. Laboratories have had to not only extend the range of measurements they perform, but also become involved in risk assessment using computer-assisted test interpretation so that clinicians can inform patients of their risk of having the disorders for which screening is being carried out.

The goal of this document is to update information on maternal serum screening (MSS) for neural tube defects (NTD) and Down syndrome (DS). NCCLS document I/LA17-A—*Assessing the Quality of Systems for Alpha-Fetoprotein (AFP) Assays Used in Prenatal Screening and Diagnosis of Open Neural Tube Defects* was the first NCCLS document to outline the use of specific testing during pregnancy to assess fetal well-being. Information related to amniotic fluid analyses taken from I/LA17-A are updated and addressed in the Appendix.

3 Standard Precautions

Because it is often impossible to know what might be infectious, all patient and laboratory specimens are treated as infectious and handled according to “standard precautions.” Standard precautions are guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;17(1):53-80 and *MMWR* 1988;37:377-388). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections*.

4 Terminology

4.1 Definitions

Accuracy (of measurement) – Closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93)⁸; **NOTE:** See the definition of **Measurand**, below.