

## The Triple Test

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**Featured article:** Wald NJ, Cuckle HS, Densem JW, Nanchahal K, Royston P, Chard T, et al. Maternal serum screening for Down's syndrome in early pregnancy. *BMJ* 1988;297:883–7.<sup>2</sup>

The era of organized prenatal screening began in the mid-1970s with the discovery that maternal serum  $\alpha$ -fetoprotein (AFP)<sup>3</sup> could be used as a screening test for neural tube defects. This discovery led to work on how screening performance could be determined from AFP distributions in affected and unaffected pregnancies. To this end, the multiple of the median (MoM) was created (1) to allow for systematic interlaboratory AFP assay variation and factors such as gestational age. The multiple of the median was used in the 1977 UK Collaborative Study on Alpha-Fetoprotein in Relation to Neural-Tube Defects (2) and in a 1984 report on using low AFP concentrations in conjunction with maternal age in screening for Down syndrome (3). It was also used in our 1988 study described in the featured report. We combined AFP, unconjugated estriol, and human chorionic gonadotropin with maternal age to produce an estimate of the risk of having a Down syndrome pregnancy. This report represented the collaboration of 3 groups: St. Bartholomew's Hospital Medical College (University of London), the Foundation for Blood Research in Maine, and Brown University (Women and Infants Hospital) in Rhode Island. Our 1988 report brought together scientists from different disciplines: epidemiologists with access to a bank of serum samples routinely collected from pregnant women, reproductive endocrinologists, a pediatrician, laboratory scientists, statisticians, and computer programmers. This collaboration was born out of the friendships that arose from earlier work and out of a common endeavor to improve prenatal care. This report described what has since been called the "triple test" (or the Barts test in the UK), which was markedly better than using maternal age as an indication for a

diagnostic amniocentesis, as was the practice at the time. For a decade, the triple test was the main prenatal screening test for Down syndrome throughout the world. The report brought to the forefront the concept of likelihood ratios, the estimation of a woman's risk of having an affected pregnancy, and the method of estimating screening performance with several screening markers simultaneously. The multivariate gaussian method described in the report has been generally adopted in subsequent research in prenatal screening.

The impact of the 1988 report was important in several ways. First, it had an impact on the role of laboratories. Traditionally, laboratories reported concentrations of analytes. After the 1988 report appeared, the concentration of individual analytes became secondary to the reporting of the risk of being affected. The laboratory was interpreting the test, a task that had usually been left to the clinicians. The extension of the role and responsibility of the laboratory encountered some resistance from clinicians, but this transfer of responsibility became accepted, partly because the interpretation of results required software that was best placed in the laboratory. Second, the report introduced the concept of a risk estimate itself becoming the screening result, thereby simplifying the process of clinical interpretation. To quote from our report: "When screening with several tests simultaneously a difficulty arises because no single cut off level for each of the tests will be suitable; the cut off level for any one test will depend on the results of the others. A simple solution is to estimate each woman's risk of having a Down syndrome pregnancy as in Table III and to use this risk estimate as the screening variable in much the same way as if it were the result of a biochemical test." The risk estimate, then, is what matters, not the concentration of the component markers used to estimate the risk. Third, the report advanced the need to specify detection rates for given false-positive rates (or vice versa), essential in assessing screening performance. Fourth, it discredited the notion of a "normal range," which is of limited value and provides no indication of how a particular result is likely to be associated with an affected individual.

Although the triple test has been superseded by newer prenatal screening tests for Down syndrome, notably the quadruple, combined, and integrated tests (4) and, more recently, DNA tests in maternal plasma, the underlying concepts set out in the 1988 report re-

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<sup>2</sup> This article has been cited more than 675 times since publication.

<sup>3</sup> AFP,  $\alpha$ -fetoprotein; MoM, multiple of the median.

main valid for all the tests, both from a statistical perspective and in their application and objective assessment.

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