Maternal Serum Screening Approved Standard I/LA25-A2

Scope

- This standard specifies requirements and recommendations for maternal serum aspects of prenatal screening for neural tube defects (NTDs) and trisomy 21 (T21) (Down syndrome) and incorporates ultrasound measurements to ensure that screening methods and quality control procedures are carried out to a high standard.

Introduction

- Prenatal screening for serious fetal abnormalities has made significant advances since the 1970s, when maternal serum alpha-fetoprotein (MSAFP) started to be used as a screening test for open NTDs. The maternal serum screening (MSS) laboratory reports must be designed so that clinicians can inform patients of the risk of having an affected fetus.
- The goal of this document is to update information on MSS for NTDs and T21, and especially introduce first-trimester and integrated screening standards.

Definitions

- **Detection rate (DR):** proportion of affected individuals with positive test results
- **False Positive Rate (FPR):** proportion of unaffected individuals with positive test results.
- **Likelihood ratio (LR):** (DR/FPR). It is the number of times individuals with positive results are more likely to have the disorder for which they are being tested than individuals who have not been tested.
- **Odds of being affected given a positive result (OAPR):** Ratio of true-positives to false positives

- **Positive predictive value:** True-positives divided by the total number of the positives (true and false)
- **Sensitivity:** Synonym of detection rate
- **Specificity:** Proportion of unaffected individuals with a negative test result (It is the complement of the false positive rate)

Specimen collection

- Specimens can be collected any time of the day
- The patient dose not have to fast.
- Specimens should not be collected after amniocentesis.
- Without prolonged application of a tourniquet.
- Collect blood into an evacuated plastic tube without anti coagulant.

Specimen Handling and Preparation

- **Serum:**
- Allow the specimen to stand at room temperature for 30 to 45 minutes or until the clot has retracted.
- Specimens that are chylous or severely hemolyzed should be avoided
- Unconjugated estriol (uE3) is the least stable of the maternal serum analytes.
- Prolonged contact with red cells also increases the rate of breakdown of intact human chorionic gonadotropin (hCG), causing false evelation of beta-human chorionic gonadotropin
- Plasma in not recommended.

Sample Storage and Transportation

- Serum should be stored refrigerated until assayed or shipped.
- Storage of serum at 4^0 C for up to six days and overnight shipment does not affect the analyte concentration.
- Storage past one week should be at -20 ^{0} C for up to six months, or at -70 ^{0} C indefinitely.

Screening Markers

- MSAFP concentrations are about 25% lower in DS-affected pregnancies than in unaffected pregnancies.
- Total hCG was find to be elevated in maternal serum from DS pregnancies; concentrations are, on average, about twice as high in DS-affected pregnancies.
- Maternal serum uE3 was shown to be significantly reduced in DS pregnancies, concentrations of uE3 are about 25% lower in DS pregnancies, making this marker separation equivalent to MSAFP, but distribution of uE3 is tighter than for MSAFP, and therefore, the discrimination between affected and unaffected pregnancies is grater.

Quality control

- Satisfactory Laboratory Standard Operating Procedure
- Assays must be supported by the company for use in First Trimesters Prenatal Screening.
- Assays must be standardised against the relevant International Reference Preparation (IRP)

Reference Materials

Human Chorionic Gonadotropin

- Six preparations have now been established as the first WHO International Reference Reagents
- A study on behalf of the IFCC working group on hCG showed that commercial assays show considerable variation in their recognition of various forms of hCG, and their variability is the most important cause of method-related differences in hCG results in serum. Future harmonization and standardization efforts should be directed toward equimolar recognition of the major hCG isomers.

Reference Materials

Alpha-fetoprotein

- Diagnostic immunoassays for AFP are calibrated against first WHO IS for Alpha-fetoprotein (72/225)

Unconjugated Estriol

- There is no standard reference material for estriol assays

Inhibin A

- The WHO 1st International Reference Standard for Human Inhibin A (91/624)
- Inhibin A assays are available as an automated assay with chemiluminescent detection or as a ELISA assay format

Pregnancy-Associated Plasma Pretein-A

- The WHO Standard 78/610 was developed

Quality Control

External Quality Control

- Laboratories performing screening assays should, as part of good laboratory practice, participate in one of the presently available external quality control (proficiency testing) programs

Quality Control

Internal Quality Control

- Control material: each maternal serum analyte run should include appropriately position controls to assess the validity of the test results
- Materials provided by independent sources are recommended in addition to those provided by kit manufacturers
- Three analyte concentration are recommended to span the measuring rate
- These can be commercial controls bought in sufficient quantity to last for one year or more, or liquated samples made from pools of maternal serum

Quality Control

- Within Day CV%: 3-4
- Between Day CV%: 5-6

Quality Control

Epidemiological Quality Assessment

- It is important to use the correct median MoM values for the screening markers and to regularly check that the current median MoMs are close to those previously estimated. If they are not, the problem should be investigated and a revised median calculated for use in the screening program. Such epidemiological monitoring is strongly recommended.

Quality Control

Use of the Initial Positive Rate

- All laboratory **should routinely monitor the IPR.**
- Rates should be monitored monthly if the number of samples screened is sufficient to stablish a statistically reliable IPR (300 to 500 specimens)
- If, for example, the IPR were found to be 7%, with an expectation that is should be 3%, the laboratory should investigate the problem, it may be caused by assay shift in the normal median value or other factors such as older age population

Quality Control

Use of the Median Multiple of the Median

- For each analyte, the median MoM should be determined regularly on at least 100 patients, a larger number is recommended whenever practical
- It is expected that the median Mom will be 1.00
- The median MoM should lie between 0.95 and 1.05
- Median MoMs outside those values should lead to investigation of assay performance and possible revision of the median values used

Quality Control

Adjustment of Median value When Introducing a New Reagent Lot

- For methods with known lot-to-lot variability (>5%), new reagent lots should be compared with the current production reagent lot before use in the following way. About 40 previously tested specimens spanning the measurement range should be stored for not more than seven days at 4^0 C
- If the proportional bias is less than 5%, and the constant bias is than 5% of the mean value, changing median values is not usually necessary

Quality Control

Screening Workload

- The laboratory should test at least 100 woman per week

Quality Assessment of Sonographers

- Screening programs incorporating NT measurements should implement quality assessment in the same way as is performed with biochemical results