

REFERENCES

- 1) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda Maryland. The quality control of alpha-fetoprotein reagents and assay for the antenatal screening and diagnosis of open neural tube defects. *Clin Chim Acta.* 1980;105:9-24
- 2) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Standard - Second Edition.* CLSI document I/LA25-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.

CHM.32500 Result Cut-off Values**Phase II**

The report classifies a pregnancy as screen-positive or screen-negative for open neural tube defects, based on the MSAFP test results.

NOTE: Cut-off levels based on AFP MoM values or risk have been established to determine the screening performance (initial positive rate, detection rate). Use of these cut-off values in the laboratory report can assist the physician in making clinical decisions about pregnancy management.

REFERENCES

- 1) Wald NJ, et al. Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural tube defects. *Lancet.* 1977;i:1323-1332.
- 2) Cunningham MD, Tompkinson DG. Cost and effectiveness of the California triple-marker prenatal screening program. *Genet Med.* 1999;1(5):199-206.
- 3) American Society of Human Genetics. American of Human Genetics policy statement for maternal serum alpha-fetoprotein screening programs and quality control for laboratories performing maternal serum and amniotic fluid alpha-fetoprotein assays. *Am J Hum Genet.* 1987;40:5-82.

CHM.32600 Result Cut-off Values**Phase II**

The report classifies a pregnancy as screen-positive or screen-negative for fetal Down syndrome, based on the calculated risk.

NOTE: Cut-off values based on risk for Down syndrome have been established to determine the screening performance (initial positive rate, detection rate). Use of the cut-off values in the laboratory report can assist the physician in making clinical decisions about pregnancy management.

REFERENCES

- 1) Cunningham MD, Tompkinson DG. Cost and effectiveness of the California triple marker prenatal screening program. *Genet Med.* 1999;1(5):199-206.
- 2) American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No 77: Screening for fetal chromosomal abnormalities. *Obstet Gynecol.* 2007;109:217-27.
- 3) Wald NJ, Huttly WJ, Hackshaw AK. Antenatal screening for Down's syndrome with the quadruple test. *Lancet.* 2003;361:835-6.
- 4) Canick JA, et al. Comparison of serum markers in first-trimester Down syndrome screening. *Obstet Gynecol.* 2006;108(5):1192-9.

AMNIOTIC FLUID ALPHA-FETOPROTEIN (AFAFP)

Inspector Instructions:

 <ul style="list-style-type: none"> • Sampling of AFAFP policies and procedures • Sampling of AFAFP patient reports (results reported in MoM) • Sampling of QC logs 	 <ul style="list-style-type: none"> • How does your laboratory verify or establish acceptable median AFAFP values? • What is your laboratory's course of action when you receive an amniotic fluid sample that is visibly contaminated with blood? • What is your laboratory's course of action when an amniotic fluid has an elevated AFP?
---	---



- Select an abnormal AFAFP result and review records for the confirmatory testing performed, including QC for AChE testing

CHM.32700 Median AFAFP Values

Phase II

There are records that the laboratory has established its own median AFAFP values or verified that medians provided from another source are appropriate for the population being screened.

NOTE: Systematic biases in AFAFP assay values of up to 30% can occur when kits from different manufacturers are used. In addition, between-laboratory differences in equipment, reagents, and technique may introduce bias in assay results even when the same kit is used. These differences can be minimized by reporting results in multiples of the median (assuming that the medians are calculated using values measured on the population to be tested using the kit designed for screening). Package insert medians may be outdated or inappropriate and should only be used as a general guide as to expected medians. When computing AFAFP medians from laboratory data, samples from pregnancies with known (or suspected) neural tube or ventral wall defects should be removed. A reasonable practice would be to trim all values over 2.5 or 3.0 MoM prior to computing medians using a log-linear model (for data between 15 and 22 weeks' gestation). Medians below 15 weeks do not follow the log-linear model and alternative curve fitting is required.

Evidence of Compliance:

- ✓ Records for median value determination using a reasonable number of in-house results (eg, 100 or more) **OR** records documenting the appropriateness of median values derived from package inserts or other sources for the laboratory's population of women tested

REFERENCES

- 1) Amniotic fluid alphafetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. Second report of the U.K. collaborative study on alphafetoprotein in relation to neural tube defects. *Lancet*. 1979;ii:651
- 2) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening: Approved Guideline—Second Edition*. CLSI document I/LA25-A2 (ISBN 1-56238-749-9). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011

****REVISED** 12/26/2024**

CHM.32800 Median Value Calculation and Review

Phase II



AFAFP medians are calculated and reviewed at specified intervals.

Evidence of Compliance:

- ✓ Records of median values calculation and review at defined frequency

CHM.32900 Multiple of Median

Phase II

AFAFP results are reported in multiples of the median (MoM).

NOTE: Reporting of AFAFP results in terms of multiples of the median (MoM) simplifies interpretation at various gestational weeks, reduces the systematic between-laboratory and between-kit bias in results, and facilitates comparison of results between laboratories. Laboratories may compare their experiences with large-scale published studies much more readily when using MoM as the interpretive unit for AFP measurements.

REFERENCES

- 1) Wald NJ, et al. Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn*. 1989;9:813-829
- 2) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening: Approved Guideline—Second Edition*. CLSI document I/LA25-A2 (ISBN 1-56238-749-9). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011

CHM.33100 Dilution Control Phase II

If the analytical procedure for measurement of AFAFP requires specimen dilution, at least one amniotic fluid dilution control is processed with each analytic run of amniotic fluids.

Evidence of Compliance:

- ✓ Records of dilution control with each run

CHM.33200 Acetylcholinesterase Testing Phase II

Acetylcholinesterase (AChE) testing is performed on ALL amniotic fluids having elevated AFAFP concentrations.

NOTE: Acetylcholinesterase (AChE) testing is an essential confirmatory test for amniotic fluids with abnormal AFP results. The odds of having a fetus with a neural tube defect are considerably greater if both the AFAFP is elevated and the AChE is positive. The addition of AChE for the detection of neural tube defects will reduce the false positive rate while maintaining a high detection rate. This procedure may be performed in-house or by a referral laboratory. If fetal blood is present, acetylcholinesterase results must be interpreted with caution.

Evidence of Compliance:

- ✓ Patient reports showing AChE results, as applicable

REFERENCES

- 1) Report of the collaborative acetylcholinesterase study. Amniotic fluid acetylcholinesterase electrophoresis as a secondary test in the diagnosis of anencephaly and open spina bifida in early pregnancy. *Lancet*. 1981;ii:321324
- 2) Wald N, et al. Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn*. 1989;9:813-829
- 3) Crandall BF et al. Risks for fetal abnormalities after very and moderately elevated AFAFPs. *Prenat Diagn* 1997;17:837-841.

CHM.33300 Quality Control of Acetylcholinesterase Assays Phase II

Both positive and negative controls are included with each analytic run.

Evidence of Compliance:

- ✓ QC records for appropriate controls with each run

REFERENCES

- 1) Haddow JE, et al. Acetylcholinesterase and fetal malformations: modified qualitative technique for diagnosis of neural tube defects. *Clin Chem*. 1981;27:61-63
- 2) Report of the collaborative acetylcholinesterase study. Amniotic fluid acetylcholinesterase electrophoresis as a secondary test in the diagnosis of anencephaly and open spina bifida in early pregnancy. *Lancet*. 1981;ii:321324
- 3) Wald NJ, et al. Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn*. 1989;9:813-829

CHM.33400 Acetylcholinesterase Confirmation Phase II

Acetylcholinesterase-positive results are confirmed by addition of a specific inhibitor.

NOTE: Positive acetylcholinesterase results must be confirmed by the addition of a specific inhibitor of acetylcholinesterase, such as BW284C51.

Evidence of Compliance:

- ✓ Records of inhibitor testing for positive acetylcholinesterase results prior to reporting results

REFERENCES

- 1) Barlow RD, et al. A single method for amniotic fluid gelacetylcholinesterase determination, suitable for routine use in antenatal diagnosis of open neural tube defects. *Clin Chim Acta*. 1982;119:137-142.