P-ISSN : 2527-4627
Warmadewa Medical Journal
Available online http://ejournal.warmadewa.ac.id/index.php/warmadewa_medical_journal

WMJ (Warmadewa Medical Journal), Vol. 8, No.1, Mei 2023, Hal. 9-15

# Maternal Serum Alpha-Fetoprotein for Congenital Anomalies Screening

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**Abstract**

Screening examination for maternal, especially serum, is a non-invasive examination of the fetus's condition. During pregnancy, screening examinations are performed in women recommended for ultrasonography (USG), amniocentesis, or other obstetric interventions. There are three markers for maternal serum screening: alpha-fetoprotein, human chorionic gonadotropin (hCG), and unconjugated estriol. Maternal serum alpha-fetoprotein is useful for screening aneuploidies and neural tube defects. This examination, besides seeing congenital abnormalities, can be used for the risk of pregnancy complications such as placental insufficiency, intrauterine growth restriction, preeclampsia, and intrauterine fetal death. Laboratory methods for examining maternal serum alpha-fetoprotein are radioimmunoassay and microparticle capture enzyme immunoassay.

**Keywords:** Screening, Pregnancy, Alpha-Fetoprotein, Laboratory

## INTRODUCTION

Maternal serum for screening examination usually to detect Down Syndrome, Edward Syndrome, or neural tube defect in the fetus. This examination can be performed in the first and second trimesters combined with ultrasonography. Blood tests in pregnancy can be performed between 9 weeks and 11 weeks combined with ultrasonography between 9 weeks and 13 weeks to measure the amount of fluid on the skin behind the fetus's neck, called nuchal translucency. If the amount of fluid is more than normal indicates that the fetus can have Down Syndrome or Edward Syndrome. *American Congress of Obstetricians and Gynecologist* (ACOG) recommends screening for all low-risk and high-risk pregnant women. The examination include blood serum, non-invasive prenatal screening, amniocentesis, or chorionic villus sampling.(1) Screening in the second trimester can see an increased risk of Down syndrome, Edward syndrome and neural tube defects. Screening can be done between week 14 and week 20.(1)

## MATERNAL SERUM ALPHA-FETOPROTEIN

Alpha-fetoprotein (AFP) is an oncofetal protein with a molecular weight of 68000 Da. AFP was produced in the fetus's yolk sac and liver. AFP was produced in the 9$^{th}$ week of pregnancy, and synthesis of AFP decreased after birth. After birth, only a small portion of AFP remains. AFP is synthesized between the G1 and S1 phase cells in the growth of the fetus; because of this the hypothesized AFP affects cell growth. AFP can bind to estrogen, so it has a role in sexual differentiation and can protect the fetus from the mother's immune system. AFP reaches the maximum level at the 12$^{th}$ week of pregnancy, around 3-4 g/L and can cross placenta.(2)

Maternal serum alpha-fetoprotein (MSAFP) is a protein expressed by the embryo and transferred to the maternal circulation. Maternal serum alpha-fetoprotein can help to detect high-risk pregnancy and the measurement need additional information, such as accurate gestational age, maternal weight, race, presence of insulin-dependent

pre-gestational diabetes, family history of NTDs, and the number of fetuses. AFP is identified in the mother's serum as early as six weeks of pregnancy, and its level steadily rises to 0.05 g/mL in the second trimester. The greatest concentration (about 1 g/mL) is recorded at 32 weeks of pregnancy, and it gradually drops until the day of birth, reaching roughly 0.05-0.1 g/mL.(2,3)

This serum can detect neural tube defects, risk of preeclampsia, intrauterine growth restriction, and fetal death. Significant false-negative rate of about 25% suggests that about 25% of open NTDs are missed. In terms of false positives, when the MSAFP level is increased, amniocentesis is typically conducted to confirm amniotic fluid increases. MSAFP was discovered in 1970 as a screening parameter for open neural tube defects and in 1984, Merkatz's research found a patient with undetectable MSAFP, a fetus born with trisomy 18 and 41 patient with low MSAFP found a baby with autosomal disorder.(2,3)

The examination chart of maternal serum alpha-fetoprotein we can see in Figure 1. Pregnant women screened by MSAFP, and found 2.25 MoM (Multiples of the Median) until 3.0 MoM were suggested for reexamination and ultrasonography. Figure 1 is a chart of the MSAFP examination. In this chart cut-off MSAFP was 2.25 MoM and MSAFP less than 2.25 MoM was considered normal and was not needed for other examinations. MSAFP between 2.25-3.0 MoM were suggested for reexamination and ultrasonography examination. Ultrasonography was performed to examine the anatomy of the fetus, including the placenta, fetal abdominal wall, amniotic fluid, central nervous system, urinary tract, multiple pregnancies, fetal death, and other anomalies. Ultrasonography examination that can't explain fetal anomalies in suggest other examination and recalculation risk of anomaly and amniocentesis. An increase in MSAFP> 2.5 MoM can be associated with chromosome abnormalities, abnormal structures in the fetus's body such as *open neural tube defects (ONTDs)* or *abdominal wall defects,* and placental abnormalities. Increased MSAFP can happen without any reason and can be associated with *chorionic vilitis* or vascular placenta lesions. This lesion can cause the transfer of AFP from fetus to mother and can cause an increase in MSAFP. Unexplained MSAFP can be caused by intrauterine growth restriction, antepartum haemorrhage, spontaneous abortion, oligohydramnios, gestational hypertension, and fetal death.(4-6)

The mean MSAFP level was higher in gestational hypertension and preeclampsia. In worse conditions, the MSAFP level can reach 5.0 MoM. Besides a higher level of MSAFP, MSAFP levels less than 0.25 MoM are found in spontaneous abortion, premature birth, fetal death, and macrosomia. Pregnant women with HIV serum MSAFP depends on viral load and CD 4 cell count. (4-8)

Mechanisms of increased MSAFP level can be seen in fetal death was associated with protein transfer across the placenta. MSAFP increased in open neural tube defect probably from the cerebrospinal fluid and decreased swallowing reflex in a fetus. Placental distress in diabetic gestational can cause the transfer of AFP to the maternal circulation. Pregnant women with rhesus incompatibility can increase the synthesis of AFP and cause increased MSAFP. (4-8)

Figure 2 showed changes in maternal serum alpha-fetoprotein (AFP) concentrations due to fetal causes; A. Open spina bifida: increased AFP in amniotic fluid and maternal serum; B. Acrania: same as A; C. Omphalocele: same as A and B; D. Finnish type congenital nephrotic syndrome: same as A, B, and C; E. Placental damage: AFP normal in amniotic fluid, AFP increase in maternal serum; F. Trisomy 21: amniotic fluid AFP normal, placental AFP increase, maternal serum AFP decline


DOI: https://doi.org/10.22225/wmj.8.1.6074.9-15

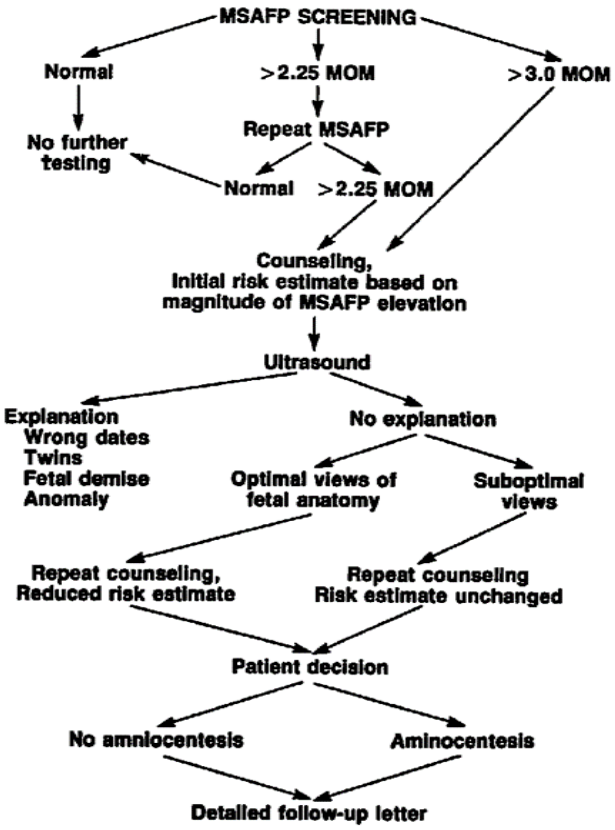


**Figure 1.** Evaluation chart of MSAFP[4].
MoM= *Multiples of the Median*

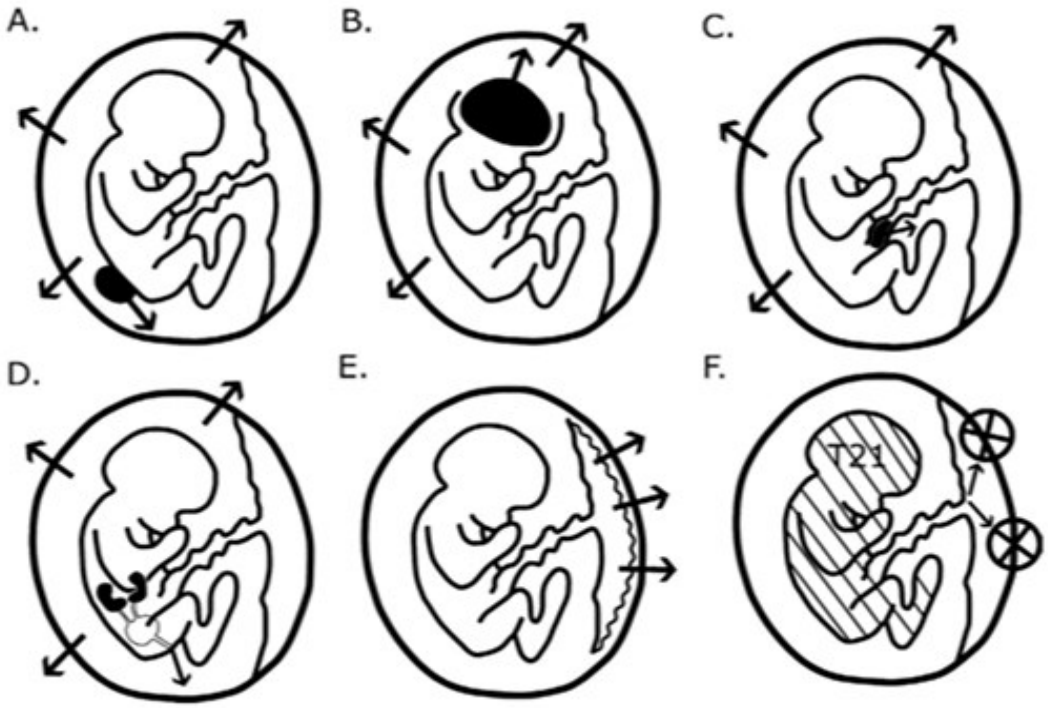


**Figure 2.** changes in maternal serum alpha-fetoprotein (AFP) concentrations due to fetal causes. (13,14)


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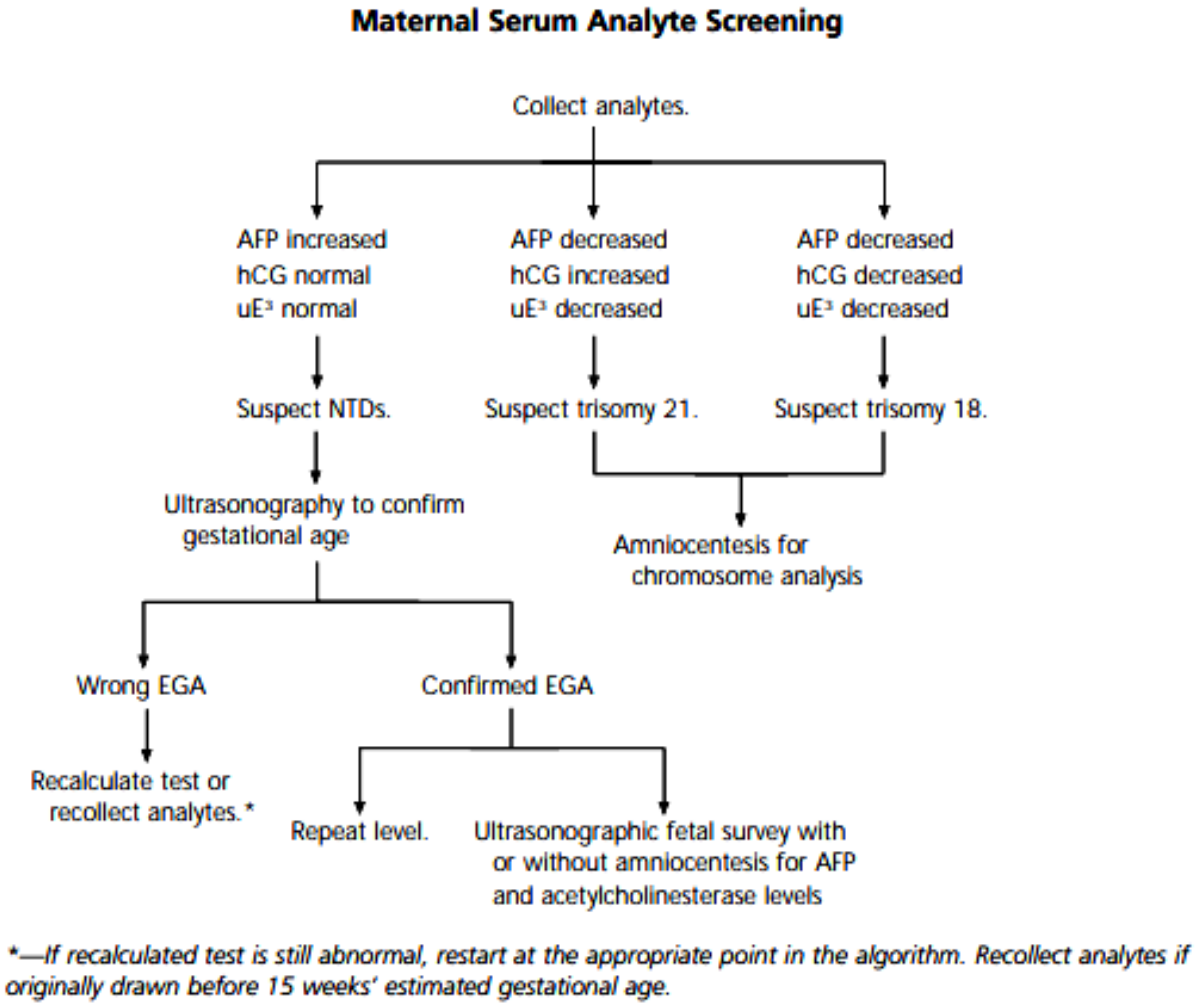


**Figure 3.** Algorithm screening pregnant women (AFP = alpha-fetoprotein; hCG = human chorionic gonadotropin; uE3 = unconjugated estriol; NTDs = neural tube defects; EGA = estimated gestational age). (15)


**Figure 4.** Principle of Radioimmunoassay (RIA). (15,16)


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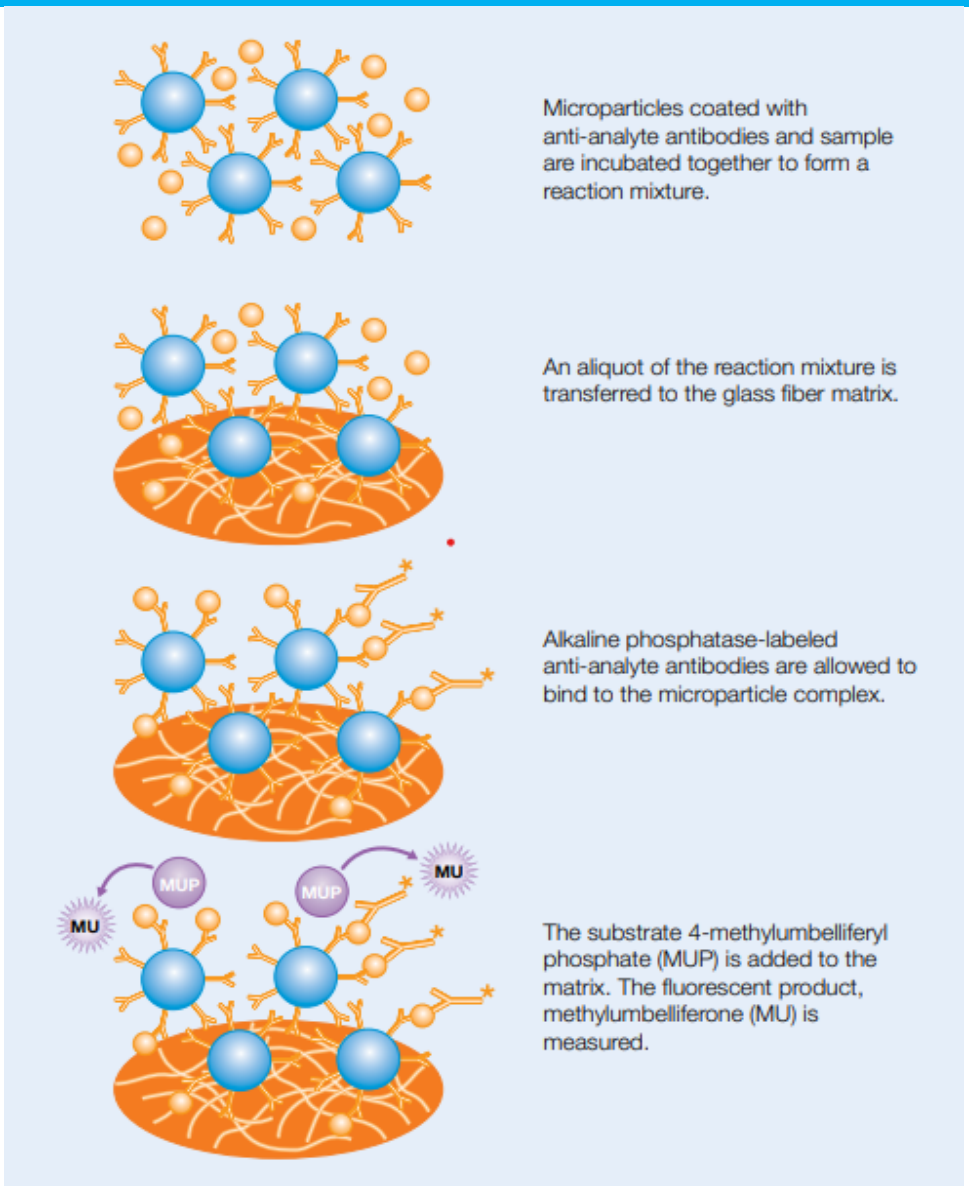


**Figure 5.** Principle of Microparticle Enzyme Immunoassay (MEIA). (15,16)

Examination combination of maternal serum alpha-fetoprotein (MSAFP) and other examinations like maternal serum human chorionic gonadotropin (MShCG) and unconjugated estriol (uE3) was best performed between 16th and 18th weeks of pregnancy but can be performed between 15th and 22nd weeks of pregnancy.

This examination is not a substitute for amniocentesis or chorionic villous sampling in high-risk pregnancies. Figure 3 shows the algorithm combination between maternal serum alpha-fetoprotein (MSAFP), maternal serum human chorionic gonadotropin (MShCG), and unconjugated estriol (uE3). Elevated AFP with normal MShCG and uE3 level was a suspect neural tube defect and ultrasound must be performed to confirm gestational age if the gestational age was wrong, the three examinations above should be repeated. The fetus was suspected of trisomy 21 if MSAFP and uE3 decreased but MShCG increased. Trisomy 18 is suspect if the three examinations above are at a decreased level. After examining the mother's serum suggest continuing with amniocentesis chromosome analysis. This combination will increase the sensitivity for detecting abnormalities in fetuses. (8-14)


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## LABORATORY METHODS

Laboratory methods for examining maternal serum alpha-fetoprotein can be carried out by radioimmunoassay and microparticle capture enzyme immunoassay. The Radioimmunoassay (RIA) used radioisotope as a label, and analyte in the patient measured a radioactivity amount. Methods of RIA can showed in figure 4. Radioimmunoassay (RIA) was divided into two which are non-competitive assay and competitive assay. The non-competitive assay shows the number of labels correlates with the amount of antigen and in the competitive assay amount of antigen shows the opposite amount of labels. A common isotope in the n RIA assay was *Gamma emitting isotope* ($I^{125}$).(15,16)

Microparticle Enzyme Immunoassay (MEIA) is an immunoassay that uses an antibody or antigen on the surface of a solid phase in small spheres called microparticles. Figure 5 shows the principle of MEIA methods. Microparticles were coated with anti-analyte antibodies, and the sample was incubated. Then reaction between the sample and the microparticle was transferred into a glass fibre matrix. Next, alkaline phosphatase is added as a conjugate and substrate fluorescent 4-Methyl Umbelliferone (MUP) and a fluorescence reaction will happen. Concentration was determined by the intensity of fluorescence proportional to the level of antigen in the sample and automatically will calculate by calibration curve and automatic tools. (15,16)

## CONCLUSION

There are some screening test in pregnancy which are ultrasound examination, amniocentesis, and serum examination. A maternal serum screening examination is a non-invasive biochemical examination to determine the state of the fetus. This examination is for a population with a high risk of congenital abnormalities. Maternal serum alpha-fetoprotein (MSAFP) is a protein expressed by the embryo and then transferred to the maternal circulation. Maternal serum alpha-fetoprotein is usually done in combination with MShCG and uE3. Detection with a combination of 3 maternal serums will increases the sensitivity. Laboratory methods for examining MSAFP are *radioimmunoassay* (RIA) dan *microparticle capture enzyme immunoassay* (MEIA).

## REFERENCE


1. Anonym. Prenatal Screening and Diagnosis. Available at: https://www.arupconsult.com/sites/default/files/Prenatal%20Screening%20and%20Diagnosis%20algorithm.pdf. Last accessed: November, 17th 2022.
2. Corapcioglu F, Turker G, et al. Serum Alpha-Fetoprotein Levels In Healthy Full-Term Neonates and Infants. Marmara Medical Journal. 2004;17(1);1-7.
3. Chasen ST. Maternal Serum Analyte Screening for Fetal Aneuploidy. Clinical Obstetrics And Gynecology.2014;57(1):182-88.
4. Richards DS, Seeds JW, et al. Elevated Maternal Serum Alpha-Fetoprotein With Normal Ultrasound: Is Amniocentesis Always Appropriate? A Review of 26,069 Screened Patients. Obstetrics and Gynecology.1988;71(2):203-7.
5. Ormond KE. Maternal Serum Screening. Journal of Genetic Counseling,1997;6(4):395-417.
6. Adinolfi A, Adinolfi M. Alpha-fetoprotein during development and in disease. Journal of Medical Genetics.1975;12:138-151.
7. MacRae AR, Canick JA. Maternal Prenatal Screening for Fetal Defects. In: Handbook Of Clinical Laboratory Testing During Pregnancy. Gronowski AM (Eds). Human Press Inc.2004:71-138.
8. Kargar et al. Evaluation of sensitivity and accuracy of maternal serum alpha fetoprotein to the diagnosis of open spina bifda in comparison with sonographic fndings of open spina bifda and Chiari II malformation in pregnant women. Gerami et al. Egypt J Radiol Nucl Med.2022; 53:177

9. Zhu et al. First-trimester maternal serum alpha-fetoprotein is not a good predictor for adverse pregnancy outcomes: a retrospective study of 3325 cases. BMC Pregnancy and Childbirth.2020; 20:104;2-8
10. Babu et al. Identification of High-Risk Pregnancies by Maternal Serum Alfa Fetoprotein Int. J. Appl. Sci. Biotechnol. 2020;8(2): 231-234
11. Cate JC, Gadsden RH. Serum Alpha-fetoprotein:II. Clinical Evaluation of Two Automated Immunoassays for Maternal Serum Screening. Annals Of Clinical and Laboratory Science. 1991;21(4):254-7.
12. Kurmanavicius et al. Pregnancy outcomes regarding maternal serum AFP value in second trimester screening. J. Perinat. Med. 2016; 1-5.
13. Kocylowski et al. Alpha-fetoprotein (AFP) — new aspects of a well-known marker in perinatology. Ginekologia Polska.2022;93(1):70–75
14. Mohamed et al. Alpha-feto protein in obstetrics. Sohag Medical Journal.2020;24(2):187-191.
15. Anonym.Maternal Serum Screening. Avaiable at: http://www3.betterhealth.vic.gov.au/bhcv2/bhcpdf.nsf/ByPDF/Maternal_serum_screening/$File/Maternal_serum_screening.pdf. Last accesed: November, 17th 2022.
16. Anonym.Learning Guide Immunoassay. Avaiable at: http://www.drtanandpartners.com/wp-content/uploads/2014/05/learning_immunoassay.pdf. Last accesed: November, 17th 2022.