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FIRST TRIMESTER SCREENING FOR DOWN SYNDROME FUNDAMENTAL CONCEPTS

The first method of screening for Down syndrome introduced in the 1970s was the use of *maternal age*. Any woman above the age of 35 was offered amniocentesis. A large number of women underwent amniocentesis and 70% of Down syndrome babies that were born to younger women were missed.

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In the late 1980s, a new method of screening was introduced that took into account not only maternal age but also the concentration of various fetoplacental products in the maternal circulation (alpha-fetoprotein (AFP), unconjugated estriol (uE3), human chorionic gonadotropin (hCG) (total and free-b). This was labeled as *triple screen*. Later inhibin-A was added and the *quadruple screen* came into vogue.

In the 90s, First Trimester Screen with the use of *maternal age and Nuchal translucency* at 11 - 13 + 6 weeks was introduced by the Fetal Medicine Foundation, UK. Subsequently, *maternal age was combined with fetal NT and maternal serum biochemistry* (free b-hCG and PAPP-A) in the first trimester. This '*combined test*' helped to significantly increase detection rates.

The focus of this booklet is to understand the following

- Down syndrome
- Concepts of screening
- First trimester screening

CHAPTER 1 - UNDERSTANDING DOWN SYNDROME

Down syndrome is a chromosomal abnormality where the child is born with an extra chromosome 21. Down syndrome occurs in about 1 in 600 pregnancies all over the world. Any woman can have a baby with Down syndrome. The risk varies with the

mother's age. 70% of babies with Down syndrome are born to younger mothers and therefore screening is offered to all women.

- All Down syndrome babies have developmental delay, the degree of which may vary. In some the developmental delay is severe while in others it may not be apparent till later.
- Cardiac defects like atrioventricular septal defects and other complex defects are seen in these children. The incidence of cardiac defects is about 50%
- 2 to 5 % of children have duodenal atresia. Another 2 percent have Hirschsprung disease
- Typical facial features are a flattened nose, small mouth, protruding tongue, small ears, and upward slanting eyes and the presence of an epicanthic fold.
- The hands are short and broad with short fingers, and may have a single palmar crease, mild incurving and short middle phalanx of the little finger. The feet may have a wide sandal gap
- Babies with Down syndrome often have decreased muscle tone at birth.
- Leukaemia occurs 20 times more often among these children

It is important to remember that not all Down syndrome children will have all the phenotypical abnormalities listed. Also, though significant structural abnormalities such as cardiac can be picked up by ultrasound, not all phenotypical abnormalities can be identified.

Maternal age and risk of Down syndrome

It is worthwhile to remember that there are many maternal age related risk tables that have been published in literature. Based on the table used, the a priori risk used in risk prediction may vary. Hence in a given practice, it is important to consistently use the same table. The Fetal Medicine Foundation has published tables with maternal age related risk of trisomy 21 at different gestational

ages. This table accounts for the natural selection of Down syndrome with advancing gestational age.

Maternal age	Trisomy 21 gestation (weeks)			
	12	16	20	40
20	1068	1200	1295	1527
25	946	1062	1147	1352
30	626	703	759	895
35	249	280	302	356
38	117	131	142	167
40	68	76	82	97

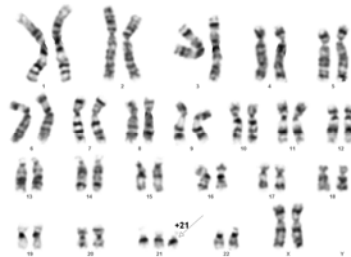
Table: Maternal age related risk as per FMF.

Cytogenetic abnormalities in Down syndrome

There are three types of cytogenetic abnormalities seen in Down syndrome. Each has a different risk of recurrence.

1. Pure trisomy 21 - This is the commonest type of Down syndrome resulting from non-disjunction of chromosomes. It occurs denovo. The risk of recurrence is low and is 1% over background risk.

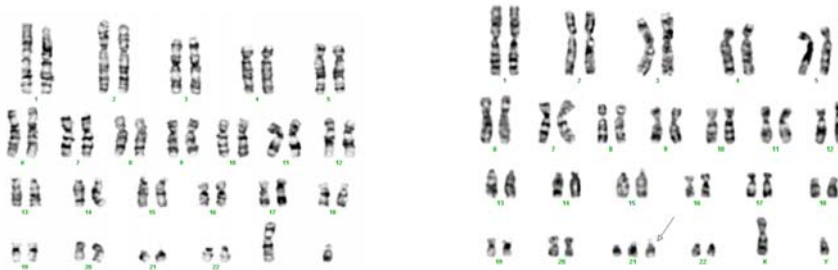
KT - pure trisomy 21



2. Mosaicism - About 2 -4% of Down syndrome are caused by mosaicism. In mosaic Down syndrome, some cells in the body have three copies of chromosome 21 and the rest of the cells have two copies of chromosomes. For example, a person might have

skin cells with trisomy 21, while all other cell types are normal. It also occurs denovo and the risk of recurrence is extremely low

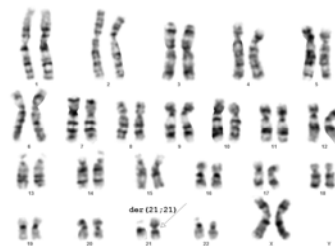
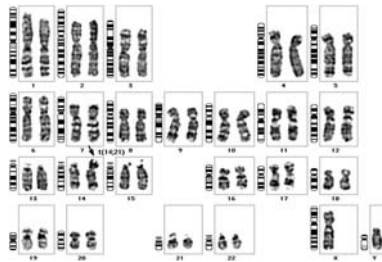
Mosaic Down syndrome - 46, XY [15] / 47, XY,+21[10]



3. Robertsonian translocation - In some people, parts of chromosome 21 fuse with another chromosome (usually chromosome 13, 14 and 21). This is called a Robertsonian translocation and occurs in 3-4% of Down syndrome. This type of translocation may occur denovo or may be transmitted from one of the parents. If it occurs denovo, the recurrence is < 1%. If one of the parent is a carrier of 13:21 or 14:21 translocation, the risk of recurrence can vary between 15- 25%; but when a parent is a carrier for 21:21 translocation, the risk of recurrence is 100%.

KT- 14:21 translocation

KT- 21:21 translocation



Therefore, it is extremely important to emphasise the need to karyotype in any child with Down syndrome, even when the diagnosis is very obvious and clear, for proper counseling in future pregnancy.

CHAPTER 2 - WHAT IS SCREENING?

Screening is a process that helps to identify people “at risk” for a condition or a problem in an asymptomatic population.

Screening is usually done for common problems and therefore needs to be applied to a large population. Ideally the condition picked up should be one where there is some treatment or intervention possible. Those who are found to be “at increased risk” of a condition may need another test to confirm if indeed the problem is present or not.

Screening may be done by identifying markers on ultrasound or biochemistry. Typically, the presence of a marker alters the baseline or a priori risk.

Baseline risk and likelihood ratios

Every individual has a baseline risk of a problem occurring. This is also called a priori risk. With respect to Down syndrome, this risk varies for each woman based on her age. For example, the baseline risk or chance that a 40 year old has a baby with Down syndrome is 1: 100.

This a priori risk is modified or changed by the result of the screening test / tests (in this case - NT / Free b-hCG / PAPP-A).

These screening tests act as filters. Each filter has a likelihood ratio, which is the number of times by which the presence of the marker increases the baseline risk. If the likelihood ratio of a test is 5, then the chance of the baby having Down syndrome increases five times. The likelihood ratio of each test varies depending on whether it is a “good” filter or a “poor” filter.

If we are applying a series of tests, then the baseline or a priori risk multiplied by the likelihood ratio of one test gives a new risk. This risk then forms the baseline risk upon which the likelihood ratio of the second test is applied. This is called as sequential screening.

What is a cut-off?

The risk cut-off is an arbitrary number or line beyond which a decision is made to offer a further diagnostic test, which is a CVS or amniocentesis. This cut-off is kept in such a fashion that the invasive testing is offered to 5% of the highest risk population. The cut-off for a particular program is dictated by policy. For example, in the UK, the current bench mark in trisomy 21 screening is a detection rate (DR) of greater than 90% of affected pregnancies with a screen positive rate (SPR) of less than 2%. The cut-off used there is 1: 100.

Currently in India, we are using a cut-off of 1:250. As and when the screening programs are well understood, standardised and established, the cut-off levels may be readjusted in the future. This can happen only if the outcome of a large number of patients who have undergone screening is used to evaluate the false positive and false negative rates. The cut-offs are set to optimise detection rates for low false positive rates, while minimising invasive procedures required.

As the efficacy of the screening test improves, the detection rate increases with a low false positive rate. This can be illustrated by the following figures which show the distribution of the measurements of a marker used for screening in unaffected and affected individuals.

Fig 1 - If the two curves do not overlap, it implies that this is a diagnostic test. Unfortunately there is no marker for Down syndrome that follows this pattern

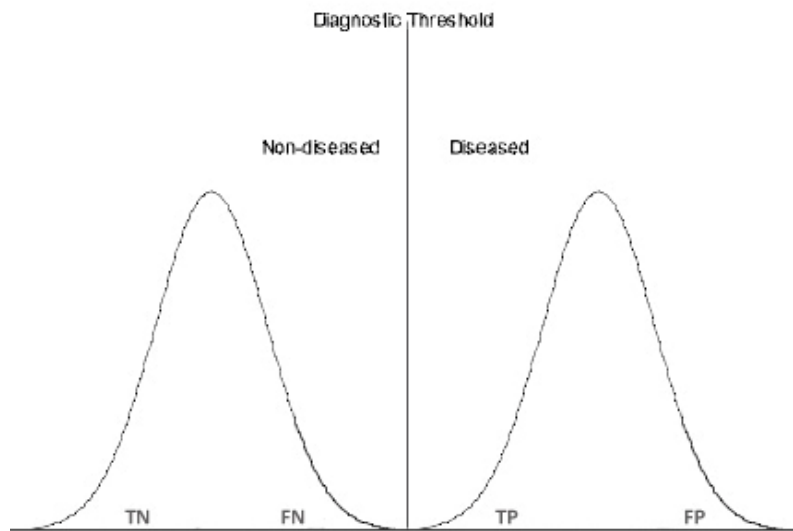


figure1

Fig 2 - All markers will have some degree of overlap as seen in this figure. The greater the degree of overlap, the poorer is the marker. The cut off value for the test is an arbitrary line that can be drawn anywhere in the overlapping area. The placement of this line will determine the false positive and false negative rates for this marker. Any false positive rate above 5% is unacceptable. Currently, screening programs are moving towards less than 3% false positive rate.

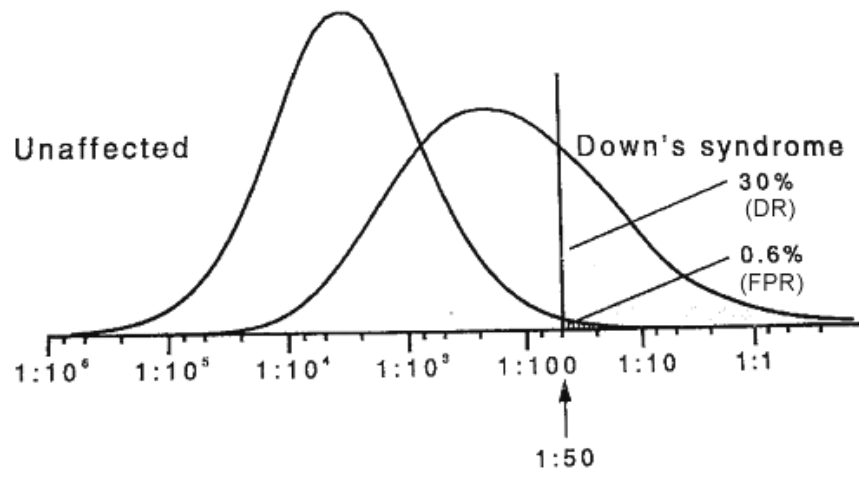


figure2a

DR – Detection rate; FPR – False positive rate

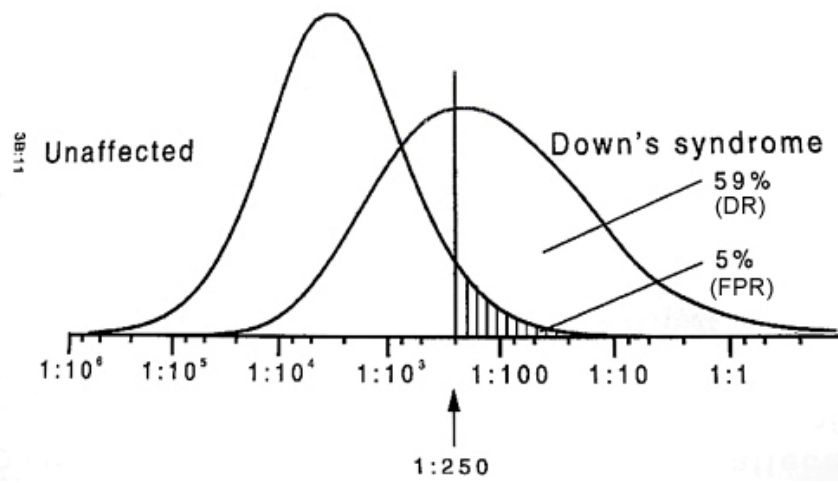


figure 2b

CHAPTER 3 - FIRST TRIMESTER SCREENING

When is it done?

The optimal time for doing a combined first trimester screening is from 11 weeks to 13 weeks 6 days. The CRL should be a minimum of 45 mm to a maximum of 84 mm.

Why 11 - 13+6?

1. Prediction of risk from NT is best from 11 weeks onwards and NT regresses by 14 weeks.
2. The biochemistry markers that are used for first trimester screening are free b-hCG and PAPP-A these are most sensitive between 9 - 11 weeks.

The process

Step 1: Maternal age related risk – A priori risk

Step 2: Measure NT correctly

Step 3: Use appropriate software for recalculating the risk for a specific NT. If Biochemical testing is also done (free beta-hCG & PAPP-A), incorporate it into the software and predict combined risk

The process of screening involves taking note of some maternal criteria. These include

- Maternal age (date of birth)
- Maternal weight
- Parity
- Method of conception (natural/ assisted)
- Ethnicity
- Smoking

At the 11 - 13 week scan the following are noted.

Accurate dating of pregnancy is the first step to the process of screening. This is either established by an earlier scan or at the time of the 11 - 13 weeks scan by taking an accurate CRL. As

interpretation of NT is based on CRL, it is important that the CRL is taken in the standard position and the baby is not too flexed or extended when the measurement is taken.



figure: flexed fetus wrong CRL



figure: neutral position correct CRL

NUCHAL TRANSLUCENCY

Nuchal translucency is the sonographic appearance of subcutaneous accumulation of fluid behind the fetal neck in the first trimester of pregnancy. The term translucency is used, irrespective of whether it is septated or not and whether it is confined to the neck or envelopes the whole fetus. The incidence of chromosomal and other abnormalities is related to the size, rather than the appearance of NT.

Fetal NT increases with gestation. For given CRL, each NT gives a likelihood ratio. Each NT value for a specific CRL has a likelihood ratio (LR) and larger the NT, higher the LR. An NT > 3.5mm is above the 99th centile and warrants direct testing.

The Nuchal translucency measurement is to be taken according to the FMF specifications.



figure: increased NT

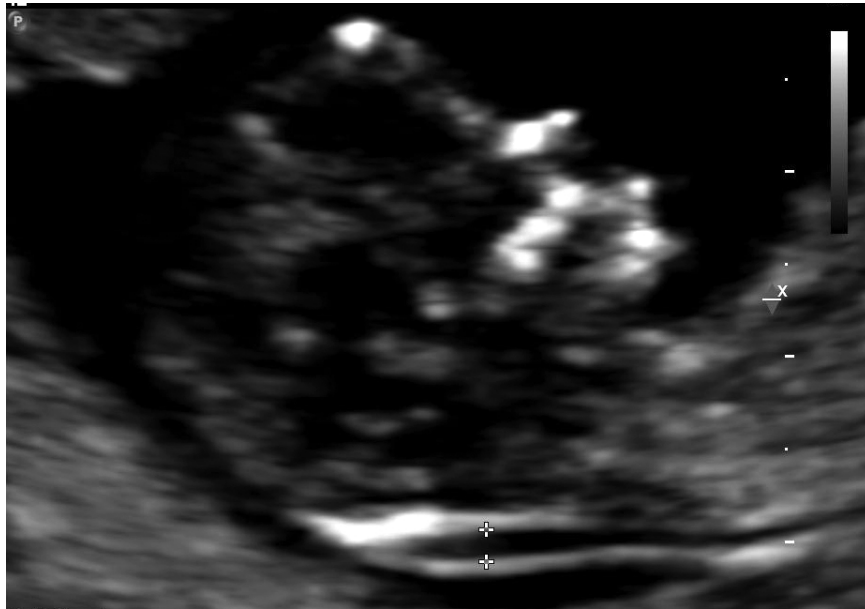


figure: NT measurement

Nuchal Translucency Measurement

- A mid-sagittal section of the fetus should be obtained and the NT should be measured with the fetus in the neutral position.
- Only the fetal head and upper thorax should be included in the image. The magnification should be as large as possible and always such that each slight movement of the calipers produces only a 0.1 mm change in the measurement.
- The maximum thickness of the subcutaneous translucency between the skin and the soft tissue overlying the cervical spine should be measured. Care must be taken to distinguish between fetal skin and amnion.
- The calipers should be placed on the lines that define the NT thickness – the crossbar of the caliper should be such that it is hardly visible as it merges with the white line of the border and not in the nuchal fluid.
- During the scan, more than one measurement must be taken and the maximum one should be recorded.

Biochemistry

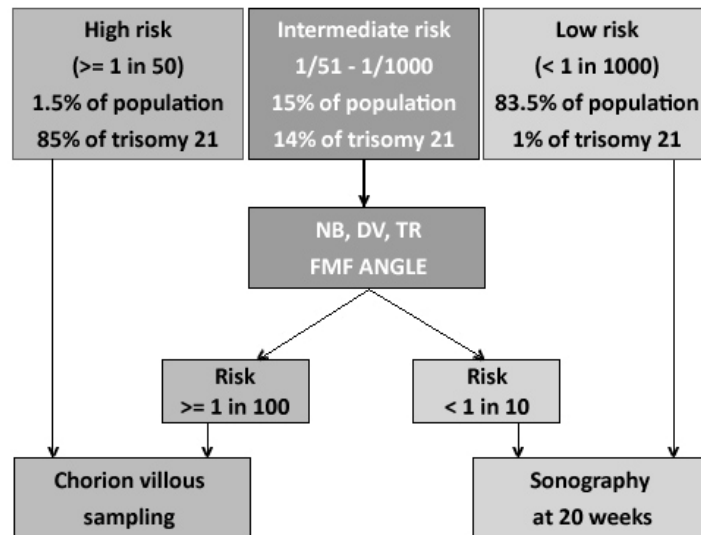
This involves a simple blood test which measures the free beta-hCG and PAPP-A. The level of free b-hCG in maternal blood normally decreases with gestation. The level of PAPP-A in maternal blood normally increases with gestation. In trisomy 21 pregnancies free b-hCG is increased, and PAPP-A level is decreased.

For a given gestation, each b-hCG and PAPP-A level represents a likelihood ratio that is multiplied by the priori risk to calculate the new risk. The higher the level of b-hCG and the lower the level of PAPP-A the higher the risk for trisomy 21.

Use of additional markers

- These markers are used to predict risk of Trisomy 21 in the boderline risk category (defined as a risk of 1: 51 to 1: 1000) and include Nasal bone - absent in 60 - 70% trisomy 21
- DV / TR - Ductus venosus abnrnalities are seen in 80% of Trisomy 21 and 5% of normal fetuses.

The FMF algorithm



The next step

When a result of screening comes back as screen negative, no further testing is generally warranted because it suggests a 'low risk' of having a baby with Down syndrome. When a woman screens positive, it does not mean that the baby is affected. This only puts her in a higher risk group and further invasive testing is offered for fetal karyotyping.

Advantage of First trimester screening

The advantages of first-trimester screening extend beyond the observed higher detection rates. The main advantage for the majority of women is the earlier reassurance provided to those with 'low-risk' results. Those who are screen positive can have a CVS and diagnosis by the 14th week. Furthermore, for those who choose to terminate an aneuploid pregnancy, decisions can be made early and social issues can be handled better. Complication rates are also lower at earlier gestations.

Advantages of the 11 - 13 week scan

The first-trimester nuchal translucency scan offers several additional benefits.

A range of abnormalities other than aneuploidy have been associated with increased nuchal translucency. These include cardiac anomalies, diaphragmatic hernia, skeletal dysplasia, and abnormal lymphatic drainage associated with neuromuscular disorders. Therefore, identification of an increased nuchal translucency measurement should prompt not only a diagnostic test for aneuploidy, but also a thorough anatomic survey for structural anomalies and a detailed fetal echocardiogram in the case of a normal karyotype.

A significant number of anomalies can be picked up at this gestational age. With the increasing use of higher resolution machines a detailed anatomical survey can be done at the 11- 13 week scan.

CHAPTER 4 - COUNSELING

Counseling forms an integral part of any screening process. A detailed explanation of the screening process is important for couples to understand what the purpose of the test is. It also helps them understand and follow up if they screen positive.

In our country the baseline awareness of Down syndrome is low and that for the screening test even less. Counseling needs to be one on one, ensuring that the information is understood correctly. Use of information leaflets is useful. In our country, the social structure is such that often counseling, unlike in the west, involves talking to the entire family and not just the couple. When we counsel we need to take on board their attitudes to termination and having a baby with Down syndrome. We also need to explain the time frame for the results.

Explained below are some of the facts that we need to cover when counseling couples for FTS

Pre-test counseling

- When counseling a couple we need to establish their awareness and understanding of what Down syndrome is and what these babies suffer from what the mother's age related risk is.
- Explain the process of screening - that it involves a scan and a blood test and that the NT and biochemistry with maternal parameters are then used by appropriate software to give an estimate of risk, that the test can only give a probability or estimate of risk. It is not a "yes" or "no" test that definitively tells us if the baby is affected or not. Explain what the result will imply if screen negative - no further testing as risk is low. If screen positive will be offered invasive testing and a fetal karyotype to confirm or rule out Down syndrome.

Post-test counseling

Once the screening is done, the result is again explained. If screen positive, the details of invasive testing are discussed and appointment for the same scheduled.

When do we offer direct invasive testing?

In general, direct invasive testing is offered in the following situations

- Maternal age > 37yrs, when the baseline risk of having a Down syndrome baby is high enough to justify invasive testing with its potential risk of pregnancy loss.
- Previous affected baby, where the couple will want a definitive answer
- When an invasive test is being done in any case for some other reason
- When we offer direct testing, we need to include the possible pregnancy loss rate from CVS and amniocentesis in our counseling. We also need to discuss the availability of FISH and formal KT, the time frame for these reports and costs, with the couple.

What should we look for when we see a report on FTS?

All Obstetricians and personnel who are involved with FTS must know what to look for when they see an FTS report. They must ensure that the test is done in the right time, in the right fashion, by people and labs who are trained and certified for the process.

This is extremely important as a wrongly or badly done test will give an inaccurate result. This will give a false sense of security to a patient or result in unnecessary invasive testing.

Check list

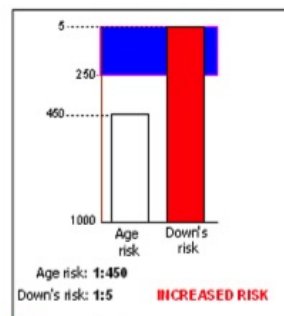
- Look at the CRL measurement, ensure dates are correct
- Look at the image of NT. If the NT is measured in a low end machine, without adequate zoom do not accept that NT.
- Biochemistry -Look to see if the values are given in MoM's. Values given without MoM's are unacceptable.
- Check if the risk prediction has included the NT + biochemistry and the combined risk has been predicted using appropriate software
- The report should mention both the woman's age related risk and give the estimated risk.

Sample reports

SCREEN POSITIVE - RESULT

Test:	Concentration:	MoM:	Corrected MoM:	Median:
HCGb	54.39 ng/mL	1.19	1.26	45.90
HT	3.50 mm	2.81	2.81	1.24
PAPP-A	2088.00 mIU/L	1.08	1.29	1937.63

DOWN'S SYNDROME (term)



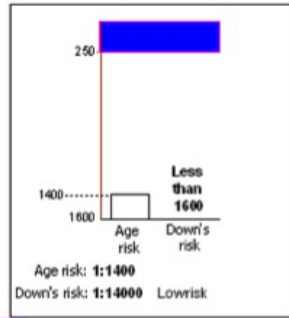
■ = Increased risk area

INTERPRETATION : SCREEN POSITIVE
Down's Risk : 1:5 in this pregnancy

SCREEN NEGATIVE - RESULTS

Test:	Concentration:	MoM:	Corrected MoM:	Median:
HCGb	20.25 ng/mL	0.59	0.53	34.10
HT	1.90 mm	1.24	1.24	1.54
PAPP-A	2324.13 mU/L	0.72	0.68	3212.23

DOWN'S SYNDROME (term)



INTERPRETATION : SCREEN NEGATIVE
 Down's Risk : **1:14000** in this pregnancy

CHAPTER 5 - SETTING UP A SCREENING PROGRAM IN YOUR PRACTICE / HOSPITAL

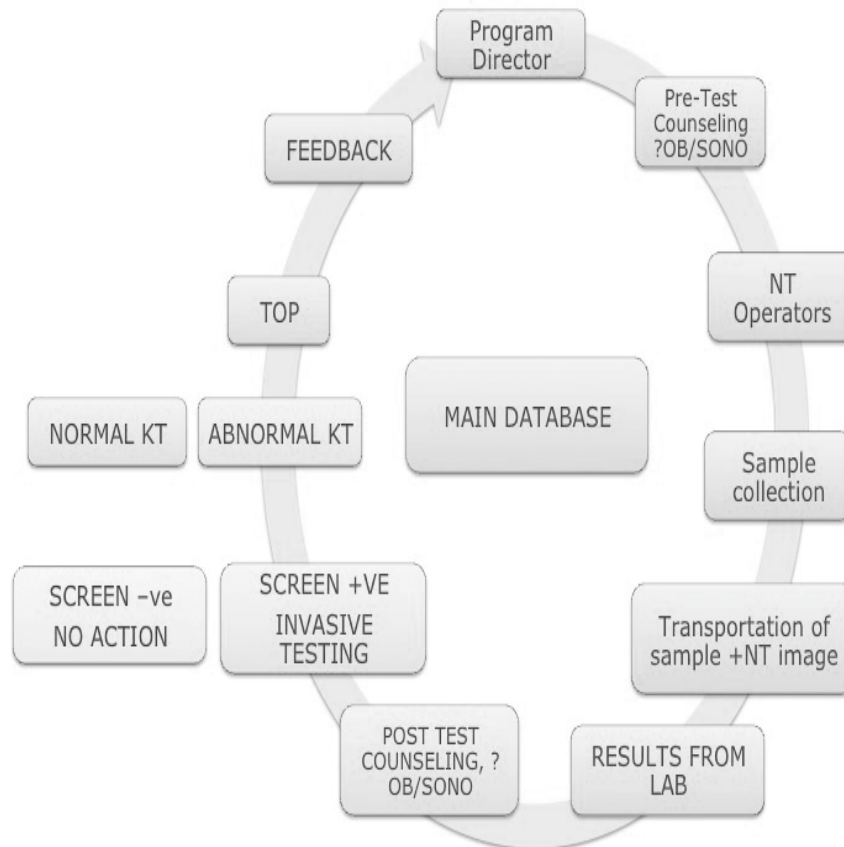


Figure: The workflow for setting up a screening program

Decide on screening test and model

Different screening processes are available and it is preferable to have a national policy that is universally adopted. Failing this, every unit has to decide on the process that it chooses to adopt and consistently stick to it.

The detection rates for the various screening modalities when performed in a guideline based and audited program areas follows.

Table: Comparison of the detection rates (DR), for a false positive rate of 5%, of different methods of screening for trisomy 21.

Method of screening	DR%
Maternal age (MA)	30
MA & maternal serum biochemistry at 15-18 weeks	50-70
MA & maternal nuchal translucency (NT) at 11-13+6 weeks	70-80
MA & fetal NT and maternal serum free b-hCG and PAPP-A at 11-13 weeks	85-90
MA & fetal NT & fetal nasal bone (NB) at 11-13+6 weeks	90
MA and fetal NT and NB maternal serum free b-hCG and PAPP-A at 11-13 weeks	95

Training and certification is an essential part of setting up a screening service. The Fetal Medicine Foundation has made it simple to take an online course followed by practical training to obtain such certification. This information is available at www.fetalmedicine.com

Responsibility of the stake holders in the screening program

Obstetrician -

- Must advocate universal screening
- Must ensure that the tests are done with appropriately certified personnel
- Must provide feedback regarding the outcomes of the pregnancies that have undergone screening.

Service provider - Sonologist / fetal medicine specialist

- Must be certified and perform the scan as per standard in every patient. *Remember certification is not an end point in itself.* It is only a declaration of competence and does not imply that every case is properly done.
- Periodic image audit is to be done

Service provider - laboratory

- Must use the approved equipments and kits for the testing. Periodic replotting of medians must be done
- Must participate in external quality control programs on an ongoing basis

If there are further queries, please feel free to contact us at ***ftsmediscan@gmail.com***