**Source of data**

After confirmation of 15 to 20 weeks of pregnancy, subsequent counseling will be done by which **preliminary data of patient** like indication, health profile, and family medical history and relevant medical information will be obtained. Samples will be collected or obtained after consideration of **high risk factor**.

The present study is based on 500 to 700 samples to be collected in-house or to be received from outside at **SRL Diagnostic Lab, A-11, Elco Arcade, Hill road, Bandra-W, Mumbai** generally referred by doctors.

**Sample collection**

Genetic amniocentesis is done in 15 to 20 weeks of pregnancy. The amniotic fluid volume at this stage is about 200 ml, out of which 20 ml of fluid is required for the studies and can be easily obtained (Purandarey, 2000). As the fluid is received in the laboratory, the demography, the quality and quantity of the sample is checked. The next step is of setting for culture. For karyotyping the chromosomes must be isolated from cells in metaphase. To get metaphase, amniotic cells must go under mitosis cell division process. Metaphase is the stage of cell cycle in which the chromosomes assume their characteristic condensed and discrete shape.

**Sample Processing**

Subsequent processing of sampling involves **tissue culturing, harvesting, slide preparation to obtain metaphase and GTG banding**. After final preparation of slide, it will be analyzed to obtain results. The turnaround time will be approx 3 weeks per sample. As such, the total of **500 to 700** samples, (considering the referral at SRL Diagnostic) will be obtained, processed, analyzed in a period of approx **2 years** (Verma, 1995)

In the current study, a 450 band level karyotyping technique will be used. The most commonly used method, i.e., Giemsa Trypsin Banding (Verma, 1982) will be adopted for the analysis,
which will be performed by using automated karyotype system using the light microscope with the magnification of 1000X.

The standard method of evaluation of the study will be adopted, that will include analysis of 20 cells and karyotyping of three cells. If abnormality is observed in any of the cells, showing only one single chromosome, additionally 20 cells will be counted to confirm the mosaicism.

The standard procedure for amnio culture, harvesting and slide preparation is as follows,

**Amniotic fluid culture flask method** (Williams, 1994)

1. Dispense collected amniotic fluid sample in sterile centrifuge tubes.
2. Centrifuge at 800-1000 rpm for 10 minutes.
3. In the laminar flow hood prepare the flask and label it with patient name, number, and date.
4. After centrifugation remove the supernatant, leave 0.5ml of fluid over the pellet.
5. Tap gently with fingers, break up the pellet and add 4 ml of culture medium.
6. Remove the medium with cells by sterile pipette and transfer in the flask.
7. Transfer flask to the CO$_2$ incubator, loosen the cap and leave it undisturbed at 37$^\circ$C for 7-8 days.
8. After 7-8 days examine the flask under inverted microscope.
9. If sufficient growth of colony is observed, feed the flask with 4ml of fresh medium.
10. After 24 hrs, flask is ready for harvesting.

**Harvesting** (Verma, 1995)

1. Add 0.1 ml of colchicines to the flask 1 hour before harvesting.
2. Remove the medium containing colchicines from the flask and place in the labeled centrifuge tube.
3. Add 2 ml of Trypsin-EDTA solution to the flask and leave it in incubator at 37$^\circ$C for 3 minutes.
4. When the cells are detached, with glass pipette aspire gently and transfer the solution to the centrifuge tube.
5. Centrifuge at 800-1000 rpm for 8 minutes.
6. Decant the supernatant and add 5ml of hypotonic solution, mix well, cover the tube tightly and incubate in the water bath at 37°C for 12 to 15 minutes.
7. After 12-15 minutes add 6-8 drops of fixative and mix gently.
8. Centrifuge the tube at 800 rpm for 10 minutes.
9. Decant supernatant, add fresh fixative 5-6 ml and mix well.
10. Repeat step no. 8 and 9 for two washes.

Slide preparation. (Verma, 1995)

1. Decent supernatant, add 0.5 ml of fresh fixative, mix well.
2. Drop 2-3 drops of the cell suspension on the slide.
3. The droplets should burst and spread evenly on the slide.
4. Drain off the remaining fixative.
5. Wipe the back of the slide and label carefully.

Age the slides in incubator at 60°C to 70°C overnight. The slides are ready for banding.
There are several methods available for banding chromosomes. The most common and routine method used is GTG banding. In this method, trypsin is used to digest the protein following which the slides are stained with giemsa. Chromosomes banding by this method gives pattern of light and dark bands, this allows identification of abnormalities in the number or morphology of chromosomes (Verma, 1982).

Analysis and final data collection
A standard karyotype with good banding resolution of 350 to 450 band level will be achieved for study (Shaffer, 2009). Nomenclature for chromosomal aberration, numerical or structural will be done by ISCN method (Felix, 1995).
After subsequent analysis compilation of comprehensive data will be correlated with the referral risk factors. All the data will be segregated and final results confirming chromosomal anomalies will be authenticated and justified. (Verma, 2003)

The unexpected results in prenatal diagnosis, which will not be expected with the referral indication, will be studies for clinical outcomes. (Kaur, 2010)