PRENATAL SCREENING FOR CHROMOSOMAL DEFECTS

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1. PRENATAL DIAGNOSIS AND GENETIC COUNSELLING

Prenatal diagnosis aims to detect congenital defects in utero. A congenital defect is defined as any abnormality in morphological, structural, functional, or molecular development present at birth, although it may appear later in life.

Prenatal diagnosis includes the screening of chromosomal defects in low-risk pregnancies or genetic counselling in high-risk pregnancies, as well as the performance of invasive diagnostic procedures in order to perform genetic tests.

The aim of Genetic Counselling or Reproductive Counselling is to determine the risk of an adverse reproductive outcome and to offer counselling and measures for its prevention. The process of pre- or post-conception Genetic Counselling includes the following sections:

- Identification of the specific cause and/or maternal-fetal reproductive risk through the collection and appropriate interpretation of family, personal and reproductive history.

- Accurate information on the risks of fetal anomaly, as well as the mechanisms of interference, with transmission of this information to the couple in a comprehensive approach, trying to reduce the anxiety associated with the screening process.

- Assessment of the benefits and limitations of the diverse alternatives to the couple, in a non-directed approach.

- Establishment of a primary or secondary prevention plan.

2. RISK OF RECURRENCE OF CHROMOSOMAL DEFECTS

The risk of recurrence of a chromosomal defect (in the same pregnant woman) has traditionally been estimated at 1 % and therefore invasive testing was always recommended. Nowadays, the risk of recurrence can be estimated more accurately.

- **For any previous chromosomal abnormality**, specific counselling on the risks of the current pregnancy should be carried out at a Genetic Counselling visit. In general, first trimester combined screening should be requested, adding the possibility of cell-free DNA or an invasive procedure depending on the previous abnormality.

- **In cases with a previous Down syndrome**, a combined first trimester screening should be performed, adding the excess risk of trisomy 21 (T21). This excess risk decreases according to the maternal age at the time of the previous trisomy 21 pregnancy. Thus, a previous gestation with trisomy 21 up to the age of 27 years will imply a greater risk in a subsequent gestation. On the

contrary, a previous trisomy 21 at an advanced maternal age will have a marginal impact on the risk of trisomy 21 for a subsequent gestation. The calculator https://appsjuan.shinyapps.io/myhgcapp/ can be used to calculate the increased risk. The fixed excess risk (independent of maternal age) applied in most calculation software programmes should not be used.

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regnant women with another previous trisomy: there is an excess risk of heterotrisomy after either a viable autosomal trisomy, a non-viable autosomal trisomy, or a sex trisomy. The excess risk corresponding to the maternal age at the time of the trisomy will be added to the combined screening risk. The calculator https://appsjuan.shinyapps.io/myhgcapp/ can be used.

	Homotrisomy		Heterotrisomy	
Maternal age (at the moment of the previous affected pregnancy)	**Risk for 1000 pregnancies**	**Excess risk 1/X**	**Risk for 1000 pregnancies**	**Excess risk 1/X**
20	4.9	204	3.7	272
21	4.9	204	3.7	272
22	4.8	208	3.6	278
23	4.7	211	3.5	284
24	4.6	218	3.5	290
25	4.5	222	3.4	296
26	4.3	234	3.2	310
27	4.1	243	3.1	325
28	3.8	264	2.9	351
29	3.5	288	2.6	381
30	3.2	316	2.4	417
31	2.8	362	2.1	476
32	2.3	436	1.7	580
33	1.9	527	1.4	702
34	1.5	666	1.1	889
35	1.2	844	0.9	1111
36	0.9	1151	0.7	1481
37	0.6	1582	0.5	2222
38	0.5	2110	0.4	2667
39	0.4	2532	0.3	3333
40	0.3	3165	0.2	4444
41	0.2	4219	0.2	6667
42	0.2	6329	0.2	6667
43	0.2	6329	0.2	6667
44	0.2	6329	0.2	6667
45	0.2	6329	0.2	6667
46	0.1	12658	0.1	13333
47	0.1	12658	0.1	13333
48	0.1	12658	0.1	13333
49	0.1	12658	0.1	13333
50	0.1	12658	0.1	13333

Excess risk of trisomy 21 following trisomy 21 based on maternal age during the affected pregnancy (homotrisomy). The last two columns of the table show the added risk of trisomy 21 after a different trisomy, according to the maternal age at the time of the affected pregnancy (heterotrisomy).
(Grande M, et al. Matern Fetal Neonatal Med. 2016 Sep 21:1-3. doi: 10.1080/14767058.2016.1219990.)

The resulting risk of T21 should be classified in the same categories as those defined for first trimester combined screening.

- **Risk of recurrence of monosomy X and triploidies**: There is no increased risk of trisomy 21 or other trisomies. There is some theoretical risk of recurrence of the same anomaly due to the existence of a germline mosaicism.
- **Familial Down syndrome**: In the case of any trisomy 21 in the parents' families, the karyotype of the affected case should be checked, or the karyotype of the parent who is a relative of the case should be carried out. There will only be an increased risk in the present gestation when Down syndrome is caused by an unbalanced translocation and the parent is a carrier of a balanced translocation (rare). Otherwise, the risk is the one expected for the general population.

3. SCREENING

3.1 FIRST TRIMESTER COMBINED SCREENING

First trimester combined screening is the preferred screening method in most settings, as it has a detection rate of 90% for a false positive rate of 4% for trisomy 21. It consists of estimating the probability of the foetus being affected by Down's, Edwards' or Patau's syndrome (T21, T18 and T13) on the basis of the risk according to maternal age, modified by first trimester ultrasound and biochemical markers. It is routinely performed in all pregnant women, regardless of age, who consult before 13.6 weeks. In those who are known to be at increased risk of chromosomal defects a genetic counselling should be performed before screening.
The process consists of a maternal blood collection and an ultrasound scan, preferably in 2 different gestational periods, although in late consultations it can be performed all on the same day.

3.1.1. The blood test should be performed between 7.6 and 13.6 weeks according to the last menstrual period, preferably at 8-10 weeks. The pregnant woman does not need to be fasting, nor does she need to have undergone a previous dating ultrasound. In the laboratory, the levels of free chorionic gonadotropin (f-hCG) and pregnancy-associated placental protein A (PAPP-A) are determined. The values obtained shall be expressed in median multiples (MoM) after the ultrasound gestational age has been reported.

3.1.2. Ultrasonography shall be performed between 11.2 and 13.6 weeks (crown-rump length, CRL, 45-80), preferably at 12 weeks, in order to establish the ultrasound-estimated due date, rule out multiple gestation, and assess nuchal translucency (NT). The 50th percentile of the Robinson & Fleming (1975) table should be used for dating according to CRL.
Outside the range of 45-80 mm CRL, proceed as follows:

- CRL < 45 mm: ultrasonography shall be rescheduled. The blood test should be repeated in case it has been extracted before 7.6 s.
- CRL 81-84 mm: feasible if blood collection was performed before 14.0 weeks (up to CRL 80 mm).
- CRL > 84 mm: requires second trimester quadruple blood screening test.

CRL (mm)	GA (wks + days)		
	50th centile	5th centile	95th centile
5	6+0	5+2	6+5
6	6+2	5+4	7+0
7	6+3	5+6	7+1
8	6+5	6+0	7+2
9	6+6	6+2	7+4
10	7+1	6+3	7+5
11	7+2	6+4	8+0
12	7+3	6+5	8+1
13	7+4	7+0	8+2
14	7+5	7+1	8+3
15	7+6	7+2	8+4
16	8+1	7+3	8+5
17	8+2	7+4	8+6
18	8+3	7+5	9+0
19	8+3	7+6	9+1
20	8+4	8+0	9+2
21	8+5	8+1	9+3
22	8+6	8+1	9+4
23	9+0	8+2	9+5
24	9+1	8+3	9+6
25	9+2	8+4	9+6
26	9+3	8+5	10+0
27	9+3	8+6	10+1
28	9+4	8+6	10+2
29	9+5	9+0	10+3
30	9+6	9+1	10+3
31	9+6	9+2	10+4
32	10+0	9+2	10+5
33	10+1	9+3	10+6
34	10+2	9+4	10+6
35	10+2	9+5	11+0
36	10+3	9+5	11+1
37	10+4	9+6	11+1
38	10+4	10+0	11+2
39	10+5	10+0	11+3
40	10+6	10+1	11+3
41	10+6	10+2	11+4
42	11+0	10+2	11+5

CRL (mm)	GA (wks + days)		
	50th centile	5th centile	95th centile
43	11+0	10+3	11+5
44	11+1	10+3	11+6
45	11+2	10+4	11+6
46	11+2	10+5	12+0
47	11+3	10+5	12+1
48	11+4	10+6	12+1
49	11+4	10+6	12+2
50	11+5	11+0	12+2
51	11+5	11+1	12+3
52	11+6	11+1	12+4
53	11+6	11+2	12+4
54	12+0	11+2	12+5
55	12+1	11+3	12+5
56	12+1	11+3	12+6
57	12+2	11+4	12+6
58	12+2	11+4	13+0
59	12+3	11+5	13+0
60	12+3	11+6	13+1
61	12+4	11+6	13+1
62	12+4	12+0	13+2
63	12+5	12+0	13+3
64	12+5	12+1	13+3
65	12+6	12+1	13+4
66	12+6	12+2	13+4
67	13+0	12+2	13+5
68	13+0	12+3	13+5
69	13+1	12+3	13+6
70	13+1	12+4	13+6
71	13+2	12+4	14+0
72	13+2	12+5	14+0
73	13+3	12+5	14+0
74	13+3	12+6	14+1
75	13+4	12+6	14+1
76	13+4	13+0	14+2
77	13+5	13+0	14+2
78	13+5	13+0	14+3
79	13+6	13+1	14+3
80	13+6	13+1	14+4

Robinson&Fleming,1975 (BrJObstetGynaecol. 1975;8;702)

3.1.3. The values of the CRL and NT should be introduced into the risk calculation software immediately after the ultrasound scan, in case of a combined 2-step test, in order to personally communicate the risks to the pregnant woman. The risk calculation can be carried out in a specific consultation or by the sonographer him or herself.

3.1.4 The T21 and T18/T13 risks are estimated on the basis of the risk inherent to maternal age, modified according to the deviation of the three markers from the expected values for gestational age. In case of oocyte donation, the maternal age to be considered should be the donor one. Validated software should be used with medians calculated in the same population. The risks of T21 or T18/13 are classified into 4 levels:

-Very high: between 1/2 - 1/10.
-High: between 1/11 - 1/250.
-Intermediate: between 1/251 - 1/1100.
-Low: <1/1100

None of the values of the isolated markers should be considered as an indication for a procedure, with the exception of increased NT (> 99th percentile), that should undergo invasive testing and gestational follow-up in the Prenatal Diagnostic Unit, at least until 22 weeks (*as detailed in increased NT chapter*).

CRL (mm)	Centile								
	1	2.5	5	10	50 (median)	90	95	97.5	99
45	0.49	0.58	0.67	0.78	1.18	1.70	1.88	2.05	2.27
46	0.51	0.61	0.70	0.80	1.22	1.74	1.92	2.10	2.32
47	0.53	0.64	0.72	0.83	1.25	1.78	1.97	2.14	2.38
48	0.56	0.66	0.75	0.86	1.28	1.82	2.01	2.19	2.43
49	0.58	0.68	0.77	0.89	1.32	1.86	2.05	2.24	2.47
50	0.60	0.70	0.80	0.91	1.35	1.90	2.09	2.28	2.52
51	0.62	0.73	0.82	0.93	1.38	1.94	2.13	2.32	2.57
52	0.64	0.75	0.84	0.96	1.41	1.97	2.17	2.37	2.61
53	0.66	0.76	0.86	0.98	1.43	2.01	2.21	2.41	2.66
54	0.67	0.78	0.88	1.00	1.46	2.05	2.25	2.45	2.70
55	0.69	0.80	0.90	1.02	1.49	2.08	2.29	2.49	2.75
56	0.70	0.82	0.92	1.04	1.52	2.11	2.33	2.53	2.79
57	0.72	0.84	0.94	1.06	1.54	2.15	2.36	2.57	2.83
58	0.73	0.85	0.96	1.08	1.57	2.18	2.40	2.60	2.87
59	0.75	0.87	0.97	1.10	1.59	2.21	2.43	2.64	2.91
60	0.76	0.88	0.99	1.12	1.61	2.24	2.47	2.68	2.95
61	0.77	0.89	1.00	1.13	1.64	2.28	2.50	2.71	2.99
62	0.79	0.91	1.02	1.15	1.66	2.31	2.53	2.75	3.03
63	0.80	0.92	1.03	1.16	1.68	2.33	2.56	2.78	3.07
64	0.81	0.93	1.05	1.18	1.70	2.36	2.60	2.82	3.11
65	0.82	0.94	1.06	1.19	1.72	2.39	2.63	2.85	3.15
66	0.83	0.96	1.07	1.21	1.74	2.42	2.66	2.89	3.18
67	0.84	0.97	1.08	1.22	1.76	2.45	2.69	2.92	3.22
68	0.85	0.98	1.10	1.24	1.78	2.48	2.72	2.95	3.26
69	0.85	0.99	1.11	1.25	1.80	2.50	2.75	2.98	3.29
70	0.86	1.00	1.12	1.26	1.82	2.53	2.78	3.02	3.33
71	0.87	1.01	1.13	1.27	1.84	2.56	2.81	3.05	3.36
72	0.88	1.02	1.14	1.29	1.86	2.58	2.84	3.08	3.40
73	0.89	1.02	1.15	1.30	1.87	2.61	2.86	3.11	3.43
74	0.89	1.03	1.16	1.31	1.89	2.63	2.89	3.14	3.46
75	0.90	1.04	1.17	1.32	1.91	2.66	2.92	3.17	3.50
76	0.90	1.05	1.18	1.33	1.93	2.68	2.95	3.20	3.53
77	0.91	1.05	1.18	1.34	1.94	2.70	2.97	3.23	3.56
78	0.92	1.06	1.19	1.35	1.96	2.73	3.00	3.26	3.60
79	0.92	1.07	1.20	1.36	1.97	2.75	3.02	3.29	3.63
80	0.92	1.07	1.21	1.37	1.99	2.77	3.05	3.32	3.66
81	0.93	1.08	1.21	1.38	2.00	2.80	3.08	3.34	3.69
82	0.93	1.09	1.22	1.38	2.02	2.82	3.10	3.37	3.72
83	0.94	1.09	1.23	1.39	2.03	2.84	3.13	3.40	3.75
84	0.94	1.10	1.23	1.40	2.05	2.86	3.15	3.43	3.78

NT (mm) centiles according to CRL (Borrell et al., Progr Obstet Ginecol 2006;49:434)

3.1.5 Biochemical marker values should be corrected for several maternal characteristics: weight, ethnicity, and insulin-dependent diabetes. In pregnant women with kidney transplant, it is highly recommended that b-HCG values are corrected for creatinine levels.

3.1.6 In dichorionic twin pregnancies, the risk should be estimated for each foetus, applying the necessary correction factors for the biochemical markers. In monochorionic twins, there will be a single risk calculated according to the mean of the two NTs. In pregnancies with more than 2 foetuses, the risk should be estimated taking into account maternal age + NT of each foetus, without the use of biochemical markers (*detailed in the Multiple gestation chapter*).

3.1.7. In the case of a vanishing twin, if the embryo has a measurable CRL, the trisomy risks are calculated according to maternal age and NT (without biochemical markers). If the embryo is not present, estimated risk shall be calculated as for a singleton pregnancy.

3.1.8. When the estimated risk of trisomy 21 or 18/13 is very high (>1/10), a genetic counselling appointment should be scheduled in order to offer an invasive procedure. In cases where the results are not provided immediately to the pregnant women (either because it is performed in 2 steps or because

the laboratory is responsible for giving the results), the pregnant woman must be informed within 48 h (2 working days) after the first trimester ultrasound. At the counselling appointment, pregnant women should be assessed about the meaning of high-risk screening, the complications associated with invasive procedures, and the limitations of the various genetic studies. Blood group and serologies should be checked prior to invasive procedures. It is desirable that informed consent for invasive procedures is completed within 24 hours prior to the procedure, when possible.

3.1.9 In case of high risk (1 /11-1 / 250), a genetic counselling appointment should be scheduled to inform about the implications of high-risk screening and to explain the advantages and disadvantages of genetic testing (cell-free DNA vs invasive testing). The main advantage of cell-free DNA is to avoid the risk of fetal loss; and the disadvantage is that the information is restricted to the chromosomes studied and that it is less accurate, although detection for trisomy 21 is 99%. The disadvantage of the invasive diagnostic test is a 0.1-0.2% risk of fetal loss; and the advantage is that it provides highly accurate information on all chromosomes. The pregnant woman and her partner must then choose their preferred method.

3.1.10 In case of intermediate risk (1 / 251-1 /1100), women are offered to undergo cell-free DNA testing, after counselling about advantages and limitations.

3.2. SECOND TRIMESTER MATERNAL SERUM SCREENING

This screening method should be restricted to pregnant women who present after 14.0 weeks, as it has a lower detection rate (75%) than first trimester screening. It consists of estimating the risk for T21 and T18/T13 based on the risk inherent to the maternal age at delivery, modified by the deviation of second trimester biochemical markers. The procedure consists of a maternal blood draw.

3.2.1. A previous ultrasound scan is required to date the pregnancy and rule out multiple gestation. When NT has not been assessed in the first trimester, the nuchal fold should be assessed as an independent marker.

3.2.2. Blood should be drawn between 14.0 and 19.6, but preferably 15-18 weeks. It is not necessary for the pregnant woman to be fasting. The most effective test is the quadruple test, which includes the free fraction of chorionic gonadotrophin (f-hCG), alpha-fetoprotein (AFP), unconjugated estriol (uE3) and Inhibin-A (inhA). When Inhibin-A is not available, the triple test (f-hCG + AFP + uE3) can be performed, but the double test (f-hCG + AFP) should not be chosen. The obtained values should be expressed in median multiples (MoM) according to the gestational age determined by ultrasound.

3.2.3. T21 and T18/T13 risks are estimated on the basis of the risk inherent to the maternal age at delivery, modified according to the deviation of the markers from the values expected for gestational age. In case of oocyte donation, the maternal age to be considered is the donor one. A software validated with medians from the population itself will be used. High risk is considered when the risk is 1/250 for T21 or T18/T13. A single alteration of one marker should not be considered as an indication for a procedure.

3.2.4. Biochemical marker values will be corrected for several maternal characteristics: weight, ethnicity, insulin-dependent diabetes, and smoking habit. In pregnant kidney transplant recipients, it is recommended that f-hCG values are corrected by creatinine levels.

3.2.5 In twin gestations, correction factors corresponding to each marker are applied, knowing that it is less effective (50% detection rate). This screening method is not applicable in higher order multiple pregnancies.

3.2.6 If AFP > 2.5 MoM, a detailed ultrasound assessment should be performed to rule out neural tube or abdominal wall defects. Amniocentesis for determination of AFP and AChE in amniotic fluid may be considered in case of uncertain ultrasonography.

3.2.7. For counselling, we should apply the first trimester risk cutoffs, with the difference that amniocentesis will be offered as the preferable invasive procedure.

4. CELL-FREE FETAL DNA IN MATERNAL BLOOD

4.1 In maternal plasma, a small proportion (≈10%) of the circulating free (i.e. extracellular) DNA has fetal origin from the trophoblast. The study of this free fetal DNA allows the detection of the most frequent chromosomal abnormalities. Usually only the 3 viable autosomal trisomies (21, 18, 13) and sex aneuploidies (X, Y) are studied, but it can also be extended to trisomies of the other chromosomes (1 to 22) and selected microdeletions, with lower detection rates. Triploidies are only detected with the SNP genotyping method, but not with the relative count method, which is the most common. Cell-free DNA is a screening test, not a diagnostic method, as its positive result needs to be confirmed by invasive testing.

4.2 The blood sample is about 10 mL and can be taken from 10 weeks of gestation onwards (until delivery). It is an indication for free fetal DNA testing:

- High risk (1/11- 1/250): as an alternative to invasive testing, taking into account its advantages and disadvantages (see 3.1.9).
- Intermediate risk (1 / 251-1 / 1100)

In the case of previous aneuploidy, it could be offered as an alternative to invasive testing, weighing the advantages and disadvantages. It is preferable to perform first trimester combined screening, taking into account the increased risk because of the previous case, and act according to the resulting risk category. In the case of very high risk, free DNA testing may be an option. It is not recommended in the case of ultrasound abnormality or increased NT, as an invasive procedure should be offered.

4.3 As so far it is not a diagnostic test, it should be confirmed with an invasive diagnostic test (chorionic villus sampling biopsy or amniocentesis). In the case of T21 with or without ultrasound signs; or any other trisomy with ultrasound signs, the diagnosis can be confirmed by chorionic villus sampling. In case of other aneuploidies (non-T21), if there are no ultrasound signs, the confirmation should be performed by amniocentesis. The probability of a confirmed positive result (positive predictive value) for trisomy 21 is high (91% in high risk and 82% in low risk) and lower for trisomies 13-18 (85% in high risk and 40% in low risk). The false positive rate is low and corresponds to 0.1% for each chromosome tested.

4.4 A low risk result implies that it is very unlikely that the foetus is affected (false negative result). The detection rate for cell-free DNA is 99% for trisomy 21, 97% for trisomy 18 and 97% for trisomy 13. The risk of a false negative for trisomy 21 is extremely low (1-2%).

4.5 In a few cases (4%) a result cannot be obtained. In half of the cases this is due to an inadequate maternal blood sample and in the other half the cause is a low fetal fraction (the percentage of fetal DNA out of the total free DNA in maternal circulation is too low to obtain a reliable result, usually fetal fraction <3%). In these cases, it has been observed that there is a 2-4% risk of trisomy 18/13 or triploidy and a repeat maternal blood collection at an older gestational age or an invasive procedure may be chosen, which is particularly indicated in pregnant women with a high body mass index (BMI>30).

4.6 The results of free DNA testing are more reliable at higher fetal fractions and at higher estimated risks (either from the first trimester combined test, second trimester biochemical screening or based on maternal age in the absence of screening). There are several online calculators that customise the positive or negative predictive value of a result according to the estimated risks.

4.7 Free fetal DNA testing can also be applied in twin gestations, with the exception of some commercial kits (Clarigo). Its effectiveness in monochorionic twins should be the same as in singletons, while in dichorionic twins it is not well established, although it is thought to be high. The rate of non-informative results has been found to be almost double that of singletons, because the fetal fraction of each twin must exceed 3%. In the case of a vanishing twin, DNA from the non-evolving foetus can be found more than 8 weeks after the fetal demise. Therefore, there is a high risk of false positives.

4.8 There are few exclusion criteria for performing cell-free DNA testing, these are: pregnant women with organ or bone marrow transplantation, and vanishing twin. Ovodonation is no longer a reason for exclusion for the vast majority of commercial kits. Being overweight and heparin treatment are not exclusion criteria.

5. GENETIC SONOGRAM

Genetic sonogram is defined as the evaluation of ultrasound markers for aneuploidy in order to modify the calculated risk using the likelihood ratios (LR) of the studied markers. In cases of intermediate risk, it may be an option when cell-free DNA is not available. A genetic sonogram is also indicated when an altered marker is found by chance.

5.1 FIRST TRIMESTER GENETIC SONOGRAM

5.1.1 The first trimester genetic sonogram consists of modifying the risk of trisomy 21 of the combined screening based on the evaluation, between 11.2 and 14.2 weeks (CRL 45-84 mm), of the secondary ultrasound markers: absent nasal bone, ductus venosus with absent or reverse flow on atrial contraction, and tricuspid regurgitation.

5.1.2 When the ductus venosus is systematically studied during the first trimester screening ultrasound, and pathologic flow is found (reverse or absent flow on wave A), the other 2 secondary first trimester ultrasound markers should be evaluated, preferably during the same scan, and, if not possible, by deferring to a subsequent specific ultrasound (genetic sonogram). The T21 risk of the combined test will be modified according to the corresponding LRs and fetal echocardiography should be indicated in all cases due to increased risk of congenital heart defects.

5.1.3 In case of a single altered secondary ultrasound marker, the risk of the combined first trimester screening should be multiplied by the corresponding "Isolated LR (iLR)".

5.1.4 In case of more than one secondary marker, the risk of combined first trimester screening is multiplied by the "Positive LR (PLR)" of the markers present and the "Negative LR (NLR)" of the absent markers.

5.1.5 When all markers are negative, the resulting LR is 0.21.

	Detection rate	False positive rate	PLR	NLR	iLR
Nuchal translucency					
Rate	69% (68/99)	5.0% (549/11,014)	–	–	–
95% CI	60–78	4.6–5.4			
Nasal bone					
Rate	20% (15/77)	1.3% (108/8,506)	15	0.82	3.9
95% CI	11–28	10.5–14.9			
Ductus venosus					
Rate	54% (50/93)	5.3% (572/10,830)	10.2	0.49	4.4
95% CI	44–64	4.9–5.7			
Tricuspid flow					
Rate	49% (17/35)	3.4% (37/1,078)	14.3	0.53	5.8
95% CI	32–65	2.4–4.5			

- *(Illa, Fetal Diagn Ther 2013; 34:116-120)*

5.1.6 Risk modification of the combined test with the first trimester ultrasound markers can be done with the screening application itself, if it allows it (SsdwLab, Roche), or using the online calculator https://appsjuan.shinyapps.io/myhgcapp/

When the resulting risk of trisomy 21 is determined, the risk level should be used in the same way as for the combined first trimester screening.

5.2 SECOND TRIMESTER GENETIC SONOGRAM

5.2.1 Any pregnant woman who consults between 20.0 - 24.6 weeks without previous screening should be offered a second trimester genetic sonogram in order to estimate the risk of trisomy 21, since neither first nor second trimester screening can be performed.

5.2.2 The following 9 T21 sonographic markers should be assessed: hypoplastic or absent nasal bone (≤2.5 mm), ARSA, ventriculomegaly (≥10 mm), increased nuchal fold (≥6 mm), intestinal hyperechogenicity (similar to bone), pyelic dilatation (≥4 mm), intracardiac hyperechogenic focus, shortened humerus (< 5th percentile) and shortened femur (< 5th percentile) (see Prenatal ultrasound screening chapter).

5.2.3 The risk of trisomy 21 inherent to maternal age is considered:

COMPLETED YEARS	MONTHS 0	1	2	3	4	5	6	7	8	9	10	11
18	1:1561	1:1560	1:1559	1:1558	1:1557	1:1556	1:1556	1:1555	1:1554	1:1553	1:1552	1:1551
19	1:1550	1:1549	1:1548	1:1547	1:1546	1:1544	1:1543	1:1542	1:1541	1:1540	1:1538	1:1537
20	1:1536	1:1534	1:1533	1:1532	1:1530	1:1529	1:1527	1:1526	1:1524	1:1523	1:1521	1:1519
21	1:1518	1:1516	1:1514	1:1512	1:1510	1:1508	1:1506	1:1504	1:1502	1:1500	1:1498	1:1496
22	1:1494	1:1492	1:1489	1:1487	1:1485	1:1482	1:1480	1:1477	1:1474	1:1472	1:1469	1:1466
23	1:1463	1:1461	1:1458	1:1455	1:1452	1:1448	1:1445	1:1442	1:1439	1:1435	1:1432	1:1428
24	1:1425	1:1421	1:1417	1:1414	1:1410	1:1406	1:1402	1:1398	1:1394	1:1390	1:1385	1:1381
25	1:1376	1:1372	1:1367	1:1363	1:1358	1:1353	1:1348	1:1343	1:1338	1:1333	1:1328	1:1322
26	1:1317	1:1311	1:1306	1:1300	1:1294	1:1289	1:1283	1:1277	1:1271	1:1264	1:1258	1:1252
27	1:1245	1:1239	1:1232	1:1225	1:1219	1:1212	1:1205	1:1198	1:1191	1:1183	1:1176	1:1169
28	1:1161	1:1154	1:1146	1:1138	1:1130	1:1123	1:1115	1:1107	1:1099	1:1090	1:1082	1:1074
29	1:1065	1:1057	1:1048	1:1040	1:1031	1:1022	1:1014	1:1005	1:996	1:987	1:978	1:969
30	1:960	1:951	1:942	1:932	1:923	1:914	1:905	1:895	1:886	1:877	1:867	1:858
31	1:848	1:839	1:829	1:820	1:810	1:801	1:791	1:782	1:772	1:763	1:753	1:744
32	1:734	1:725	1:716	1:706	1:697	1:687	1:678	1:669	1:660	1:650	1:641	1:632
33	1:623	1:614	1:605	1:596	1:587	1:578	1:570	1:561	1:552	1:544	1:535	1:527
34	1:518	1:510	1:502	1:494	1:486	1:478	1:470	1:462	1:454	1:446	1:439	1:431
35	1:424	1:416	1:409	1:402	1:395	1:387	1:381	1:374	1:367	1:360	1:354	1:347
36	1:341	1:334	1:328	1:322	1:316	1:310	1:304	1:298	1:292	1:287	1:281	1:275
37	1:270	1:265	1:259	1:254	1:249	1:244	1:239	1:235	1:230	1:225	1:221	1:216
38	1:212	1:207	1:203	1:199	1:195	1:191	1:187	1:183	1:179	1:175	1:171	1:168
39	1:164	1:161	1:157	1:154	1:151	1:147	1:144	1:141	1:138	1:135	1:132	1:129
40	1:126	1:124	1:121	1:118	1:116	1:113	1:111	1:108	1:106	1:103	1:101	1:99
41	1:97	1:94	1:92	1:90	1:88	1:86	1:84	1:82	1:81	1:79	1:77	1:75
42	1:73	1:72	1:70	1:69	1:67	1:65	1:64	1:63	1:61	1:60	1:58	1:57
43	1:56	1:54	1:53	1:52	1:51	1:49	1:48	1:47	1:46	1:45	1:44	1:43
44	1:42	1:41	1:40	1:39	1:38	1:37	1:36	1:35	1:35	1:34	1:33	1:32
45	1:31	1:31	1:30	1:29	1:29	1:28	1:27	1:27	1:26	1:25	1:25	1:24
46	1:24	1:23	1:22	1:22	1:21	1:21	1:20	1:20	1:19	1:19	1:18	1:18
47	1:17	1:17	1:17	1:16	1:16	1:15	1:15	1:15	1:14	1:14	1:14	1:13
48	1:13	1:13	1:12	1:12	1:12	1:11	1:11	1:11	1:11	1:10	1:10	1:10
49	1:9	1:9	1:9	1:9	1:9	1:8	1:8	1:8	1:8	1:7	1:7	1:7
50	1:7	1:7	1:7	1:6	1:6	1:6	1:6	1:6	1:6	1:5	1:5	1:5

Risk of trisomy 21 at term, according to maternal age, expressed in years and months at due date. (Cuckle et al, Br J Obstet Gynaecol 1987;94:387-92)

5.2.4 If an isolated single marker is found, age risk should be multiplied by the LR of this marker, shown in the last column of the table described by Agathokleous et al (2013).

Table 11 Pooled estimates of detection rate (DR), false positive rate (FPR) and positive and negative likelihood ratios (LR+ and LR−) of sonographic markers for trisomy 21 and estimated likelihood ratio (LR) of individual isolated markers

Marker	*DR (95% CI) (%)*	*FPR (95% CI) (%)*	*LR+ (95% CI)*	*LR− (95% CI)*	*LR isolated marker*[a]
Intracardiac echogenic focus	24.4 (20.9–28.2)	3.9 (3.4–4.5)	5.85 (5.04–6.80)	0.80 (0.75–0.86)	0.95
Ventriculomegaly	7.5 (4.2–12.9)	0.3 (0.2–0.4)	25.78 (12.85–51.73)	0.94 (0.91–0.98)	3.57
Increased nuchal fold	26.2 (20.3–33.0)	1.2 (0.7–2.2)	19.18 (11.55–31.84)	0.80 (0.75–0.86)	3.12
Echogenic bowel	16.7 (13.4–20.7)	1.1 (0.8–1.5)	11.44 (9.05–14.47)	0.90 (0.86–0.94)	1.65
Mild hydronephrosis	13.7 (11.1–17.0)	1.4 (1.2–1.8)	7.77 (6.22–9.71)	0.92 (0.89–0.96)	1.10
Short humerus	30.3 (17.1–47.9)	4.6 (2.8–7.4)	4.81 (3.49–6.62)	0.74 (0.63–0.88)	0.78
Short femur	27.7 (19.3–38.1)	6.4 (4.7–8.8)	3.72 (2.79–4.97)	0.80 (0.73–0.88)	0.61
ARSA	30.7 (17.8–47.4)	1.5 (1.0–2.1)	21.48 (11.48–40.19)	0.71 (0.57–0.88)	3.94
Absent or hypoplastic NB	59.8 (48.9–69.9)	2.8 (1.9–4.0)	23.26 (14.23–38.03)	0.46 (0.36–0.58)	6.58

[a]Derived by multiplying the positive LR for the given marker by the negative LR of each of all other markers, except for short humerus.
ARSA, aberrant right subclavian artery; NB, nasal bone.

(Agathokleous, Ultrasound Obstet Gynaecol 2013; 41:247-261)

5.2.5 If more than one marker is found, the age risk should be multiplied by the positive LRs of the markers that are present and by the negative LRs of the markers that are absent from the table published by Agathokleous et al. (2013).

5.2.6 If no marker is present, the age risk is multiplied by 0.13.

5.2.7 If, during morphological ultrasound (19-22 weeks), one or more than one ultrasound marker is found, the same LRs should be applied, modifying the estimated risk at first or second trimester screening, as required.

5.2.8 The risk modification of the combined test with the second trimester ultrasound markers can be performed with the screening application itself, if it allows it (SsdwLab, Roche), or using the https://appsjuan.shinyapps.io/myhgcapp/ calculator. If any marker is not evaluable, the Appendix S1 of https://obgyn-onlinelibrarywiley-com.sire.ub.edu/doi/full/10.1002/uog.12364 should be used. When the resulting trisomy 21 risk is ≥1/1100, the risk levels already described for the first trimester should be considered in order to perform further studies.

5.2.9 The nuchal fold should be routinely assessed in all pregnancies in which NT has not been assessed in the first trimester.

5.2.10 When isolated choroid plexus cysts are detected (without other findings), in single or multiple presentations, the risk of T18 is multiplied by a LR of 7. If the pre-ultrasound risk is 1/10000, this risk is increased to 1/1429. The risk of T21 will not be modified.

5.2.11 Markers of aneuploidy should be reported on the ultrasound report even if they do not increase the risk of T21 or T18/13 and the finding should be reported as a normal variant, if necessary.

6. QUALITY CONTROL OF NUCHAL TRANSLUCENCY

6.1 NT is the most powerful marker in combined first trimester screening and therefore it has the most weight in the resulting risk calculation. In fact, small deviations in its measurement have been correlated with significant decreases in the efficacy of combined first trimester screening. It is on these grounds that quality control of NT measurement is strongly recommended.

6.2 Reviewing the quality of the measurement from the individual ultrasound image is not feasible at the population level of screening. For this reason, statistical methods have been developed based on the comparison of the measurements made by an operator with the expected measurements. Two quantitative quality control methodologies are applied:

Retrospective evaluation of the MoMs and of the standard deviation of the log10 MoMs:
For each of the sonographers, the median of the MoMs of all of the NT obtained during a given period is calculated; in order to evaluate it, it is necessary that a minimum of 30 measurements have been performed. It is classified into 3 categories:

- 0.90-1.10 MoM: correct estimate of NT.
- <0.90 MoM: underestimation of TN
-> 1.10 MoM overestimation of NT

A deviation of this parameter correlates with the worse screening results, underestimation correlates with decreased sensitivity, and overestimation with increased false positive rates. In practice, almost no overestimations are found. Underestimations are classified as moderate (0.70-0.89 median MoM) and severe (<0.70 median MoM).

The Log10 MoM standard deviation value will be used as a guideline to assess the over- or under-dispersion in the measurements, with values between 0.08 and 0.12 being normal.

6.2.2. Prospective evaluation with the CUSUM chart: this prospectively monitors the measurements made by an operator. It has two lines, the upper one monitoring the overestimation deviations and the lower one monitoring the underestimation deviations. If the two lines remain within their limits, they indicate that there is no tendency towards s deviation. If the upper line exceeds its limit, there is a tendency to overestimate, and if the lower line exceeds its limit, there is a tendency to underestimate. It has the advantage over retrospective assessment that deviations can be corrected before the median MoM has deviated, with minimal impact on the screening results. If the screening application has this tool (SsdwLab, Roche), sonographers and/or technicians can apply it to their measurements and the unit manager will evaluate it at least once a year to detect any possible deviations and encourage their correction. In practice, almost no overestimations are found. Underestimations are classified as moderate (0.70-0.89 median MoM) and severe (<0.70 median MoM).