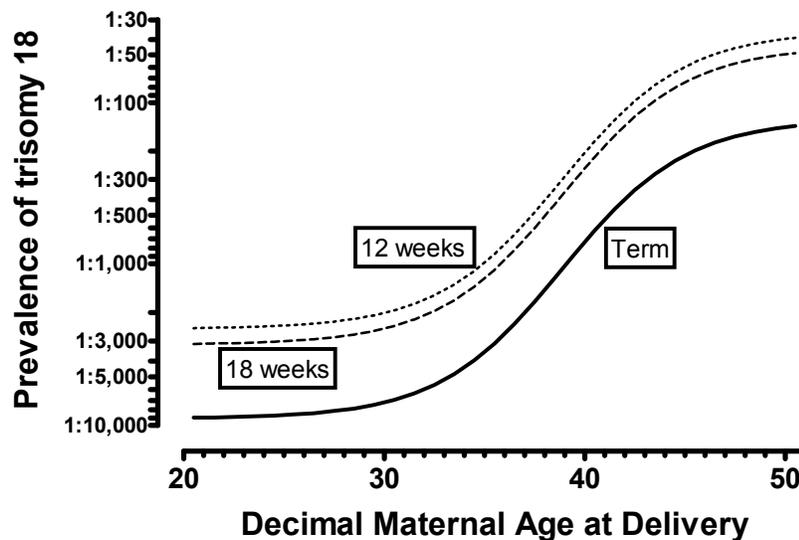


## Chapter 10. Summary

As with Down syndrome, there is a strong positive association between maternal age and increasing birth prevalence of trisomy 18, with rates of 1:8,350 and 1:994 at 25 and 40 years of age, respectively. There is also an important increase in the spontaneous rate of fetal loss, with an estimated 72%, 65% and 52% of trisomy 18 fetuses lost from about 12, 17 and 28 weeks' of gestation to term, respectively. Table 10-1 and Figure 10-1 provide these maternal age and gestational age specific results, along with the equations and values needed for computation.



**Figure 10-1. Prevalence of trisomy 18 by maternal age, at three selected times in gestation.** The solid line provides the prevalence at term. The dashed and dotted lines provide estimates at 18 and 12 weeks' gestation, respectively. Fetal loss rates of 65% and 72% were used (Morris and Savva, 2008). The prevalence (as a probability) is computed using the following equation  $p = 1/(1+\exp(a-(b/(1+\exp(c*(age-38.9))))))$ , where a, b and c are 9.11, 4.27 and -0.324, respectively (Savva *et al.*, 2010).

**Table 10-1. Prevalence of trisomy 18 by maternal age, at three selected times in gestation.**

Decimal maternal age at delivery	Prevalence of trisomy 18, expressed as an odds (1:n)		
	At Delivery	At 18 weeks	At 12 weeks
20.5	8,947	3,131	2,505
21.5	8,909	3,118	2,495
22.5	8,858	3,100	2,480
23.5	8,788	3,076	2,461
24.5	8,692	3,042	2,434
25.5	8,562	2,997	2,397
26.5	8,388	2,936	2,349
27.5	8,155	2,854	2,283
28.5	7,848	2,747	2,197
29.5	7,451	2,608	2,086
30.5	6,950	2,432	1,946
31.5	6,336	2,218	1,774
32.5	5,614	1,965	1,572
33.5	4,806	1,682	1,346
34.5	3,954	1,384	1,107
35.5	3,118	1,091	873
36.5	2,358	825	660
37.5	1,722	603	482
38.5	1,228	430	344
39.5	870	304	244
40.5	622	218	174
41.5	457	160	128
42.5	349	122	98
43.5	277	97	78
44.5	230	81	64
45.5	198	69	56
46.5	177	62	50
47.5	162	57	45
48.5	152	53	42
49.5	145	51	40
50.5	139	49	39

The most common clinical abnormalities apparent in the fetus are heart defects (e.g., ventricular septal defects) and abnormalities of the hands and feet. Among live born trisomy 18, half die within one week of delivery and only 1 in 20 (mostly females) survive the first year. All fail to thrive and all survivors will have moderate to serious mental retardation. Low birthweight is common, and fetal distress at delivery may result in unnecessary cesarean sections. It is this latter problem that makes the identification of trisomy 18 of potential benefit to the mother. Additional information can be found in Chapter 2.

Low levels of early second trimester 'triple test' markers (AFP, uE3, and hCG / free  $\beta$  subunit of hCG) have been used to assign risk for trisomy 18 for over 25 years, with detection and false positive rates of 81% and 0.4%, respectively. The algorithms have been validated by demonstration studies, and new data show the existing parameters need only minor, if any, revisions. Of interest is the finding that second trimester PAPP-A measurements are extremely low in trisomy 18 pregnancies (median MoM 0.10). These measurements are not, however, used in everyday practice, as they are not different in Down syndrome pregnancies. If they were used, a second trimester quadruple marker algorithm (triple test + PAPP-A) would improve trisomy 18 detection to an estimated 88%, while simultaneously reducing the false positive rate to about 0.1%. Table 10-2 provides expected detection and false positive rates for three combinations of tests at selected term risk cut-off levels (more complete modeling results are in Tables 3.9-2 and 3.9-3).

**Table 10-2. Modeled trisomy 18 detection rates (DR) and false positive rates (FPR) using second trimester maternal serum markers at four risk cut-off levels**

Risk at term (2 <sup>nd</sup> )	Maternal age in combination with screening markers					
	Double Test		Triple test		Quadruple test	
	AFP & hCG		Double test + uE3		Triple test + PAPP-A	
	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)
1:100 (1: 35)	38	0.27	73	0.12	81	<0.10
1:200 (1: 70)	48	0.63	78	0.26	85	<0.10
1:300 (1:105)	54	1.0	81	0.39	88	0.11
1:400 (1:140)	59	1.5	82	0.51	89	0.13

Table 10-3 provides the population parameters and truncation limits used in this modeling. These can also be recommended for assigning patient-specific risks for the second trimester serum markers. Age-related trisomy 18 risks were computed as described in Figure 10-1. The maternal age distribution used was for England and Wales in 2006 through 2008 (Figure 3.9-1). A more complete discussion of the modeling, sources of data and other markers that could be used (e.g., free  $\beta$  measurements) are found in Tables 3.8-1 and 3.8-2 and the associated text.

**Table 10-3. Modeling parameters for four second trimester maternal serum analytes in trisomy 18 and unaffected pregnancies**

Analyte	Trisomy 18 pregnancies			Unaffected pregnancies
	Median	Log mean	Log SD	Log SD
AFP	0.66	-0.1830	0.1817	0.1399
uE3	0.36	-0.4448	0.2817	0.1142
hCG	0.39	-0.4123	0.3561	0.2276
PAPP-A	0.10	-0.9871	0.3894	0.2549
<b>Pair-wise correlations</b>				
AFP	uE3		0.2501	0.1981
AFP	hCG		0.0314	0.1981
AFP	PAPP-A		0.2300	0.1536
uE3	hCG		0.0944	-0.0416
uE3	PAPP-A		-0.0413	0.0983
hCG	PAPP-A		0.1824	0.2838
<b>Truncation limits</b>				
AFP	0.33 – 2.00			
uE3	0.40 – 1.50			
hCG	0.20 – 2.50			
PAPP-A	0.20 – 1.00			

AFP = alpha-fetoprotein, uE3 = unconjugated estriol, hCG = human chorionic gonadotropin, PAPP-A = pregnancy associated plasma protein-A, SD = standard deviation

Late first trimester serum markers for trisomy 18 are also well described, but care must be taken to avoid study bias. This occurs when abnormal levels of a marker, (e.g., low PAPP-A) are used to identify high risk pregnancies that are then offered early diagnostic testing and subsequent early identification. Affected pregnancies associated with more normal marker levels would continue, but they are subject to high rates of spontaneous loss during the remaining weeks of pregnancy. The resulting over-selection of pregnancies with low PAPP-A compared to the under-selection of pregnancies with normal measures results in a bias that will overestimate the strength of association. The most commonly used markers are PAPP-A and free beta (or total hCG). However, the performance of these markers alone (70% detection at a 0.6% false positive rate) is not as good as the performance of the second trimester markers. Of some interest is one study showing trisomy 18 associated with very low uE3 measurements at this time in pregnancy, but there have been no confirmatory studies. Additional information can be found in Chapter 4.

First trimester nuchal translucency (NT) measurements are increased in pregnancies with trisomy 18. However, there are no sufficiently large, observational studies that can be used to reliably determine the population parameters. Instead, the analyses in this thesis rely on an 'unbiasing' technique using data from an ongoing clinical service. This technique randomly removed a proportion of the observations from pregnancies with 'positive' test results that were diagnosed in the first trimester, in order to properly balance these observations from pregnancies with 'negative' test results that were diagnosed at term. Applying this technique resulted in a reduction in the median NT MoM from 2.94 to 2.17. More information can be found in Chapter 5.

When these revised NT population parameters were added to the first trimester serum markers together making the 'combined' test. The resulting detection improved to an estimated 83%, with a reduction in the false positive rate to about 0.1%. Table 10-4 provides estimated detection and false positive rates for three combinations of tests at selected term risk cut-off levels (more complete modeling results are in Tables 6.2-1 and 6.2-2). These more conservative trisomy 18 modeling results are in agreement with performance reported from first trimester demonstration studies of the combined test. Table 10-5 provides the population parameters and truncation limits used in this first trimester modeling. These can also be recommended for assigning patient-specific trisomy 18 risks for the combined test. A more complete discussion of the modeling, sources of data and other markers that could be used (e.g., intact/total hCG

measurements) are found in Table 4.4-3, Figure 5.2-4 and the associated text. More information can be found in Chapter 6.

**Table 10-4. Modeled trisomy 18 detection rates (DR) and false positive rates (FPR) using first trimester serum and ultrasound markers at four risk cut-off levels**

Risk (1:n) at term (in first)	Maternal age in combination with			
	PAPP-A & free $\beta$		PAPP-A, free $\beta$ & NT	
	DR (%)	FPR (%)	DR (%)	FPR (%)
1: 50 (1:14)	56	0.4	79	0.1
1:100 (1:29)	74	0.8	86	0.2
1:150 (1:43)	80	1.4	88	0.3
1:200 (1:60)	83	2.0	90	0.4

PAPP-A = pregnancy associated plasma protein-A, free  $\beta$  = the free subunit of hCG,  
NT = nuchal translucency

**Table 10-5. Modeling parameters for three first trimester maternal serum / ultrasound markers (combined test) in trisomy 18 and unaffected pregnancies**

Analyte	Trisomy 18 pregnancies			Unaffected pregnancies
	Median	Log mean	Log SD	Log SD
PAPP-A	0.20	-0.6989	0.3207	0.2495
Free $\beta$	0.25	-0.5992	0.3255	0.2651
NT	2.17	0.3365	0.3151	0.1105
<b>Pair-wise correlations</b>				
PAPP-A	Free $\beta$		0.1286	0.1395
PAPP-A	NT		0.0000	0.0000
Free $\beta$	NT		0.0000	0.0000
<b>Truncation limits</b>				
PAPP-A				0.30 – 0.70
Free $\beta$				0.40 – 1.20
NT				0.50 – 2.00

An integrated test combines information from both the first trimester combined test and the second trimester triple test. If only serum markers (without duplication of the hCG subunit) were used, estimated detection and false positive rates would be 82% at about 0.1%, respectively. Thus, the serum integrated test is better than the second trimester serum triple test or the first trimester combined test. If first trimester NT measurements were included to form the full integrated test, detection improves to 91% at the same false positive rate, the highest performance achieved yet. Table 10-6 provides expected detection and false positive rates for serum and full integrated tests at selected term risk cut-off levels (more complete modeling results are in Tables 7.2-1 and 7.3-1). All necessary parameters have already been provided in this Chapter. More information regarding integrated testing for trisomy 18 is contained in Chapter 7.

**Table 10-6. Modeled trisomy 18 detection rates (DR) and false positive rates (FPR) using serum integrated and full integrated testing, at four risk cut-off levels**

	Serum integrated		Full Integrated	
	DR (%)	FPR (%)	DR (%)	FPR (%)
1:100 (1: 35)	76	<0.10	84	<0.1
1:200 (1: 70)	82	0.10	88	<0.1
1:300 (1:105)	84	0.16	89	<0.1
1:400 (1:140)	86	0.21	91	0.11

Serum integrated = maternal age in combination with 1<sup>st</sup> trimester PAPP-A and second trimester triple test (AFP, uE3 and hCG) measurements

Full integrated = serum integrated and 1<sup>st</sup> trimester NT measurements

Second trimester ultrasound markers have been widely studied and reported over the last three decades, but the literature is difficult to interpret. Results are confounded by the gestational age at which the ultrasound was performed, the equipment available at the time, as well as differences in training and technique among centers and sonographers. In general, a fetal anomaly scan is targeted between 18 and 20 weeks' gestation, a relatively late time in pregnancy to be determining trisomy 18 risks, when reliable and less expensive methods of identifying pregnancies at risk are widely available. This is not to say that second trimester ultrasound does not have a place in prenatal care, just that identifying trisomy 18 would not be a major factor in its routine

use. More information regarding second trimester ultrasound testing can be found in Chapter 8.

For all of these methods of assigning pregnancy risk for trisomy 18, it is necessary to offer follow-up diagnostic testing. At this time, diagnostic testing requires an invasive test, either chorionic villus sampling between 11 and 14 weeks' gestation or amniocentesis between 14 and 20 weeks' gestation. Each has an associated risk of procedure-related loss. The diagnostic tests available have expanded since the 1980s when only karyotyping was available. Now, molecular-based tests such as quantitative PCR (qfPCR) or fluorescent in situ hybridization (FISH) can be performed in a few days, with fewer resources, but with high reliability. Newer methods like array comparative genomic hybridization (array CGH) may even replace the gold standard karyotype in the near future.

In the future, it may be possible to create a diagnostic (or near diagnostic) test by relying on cell free fetal nucleic acids in the maternal circulation. Fetal sex and fetal Rh status can already be confidently diagnosed using a maternal plasma sample. Several proof of concept tests using cell free fetal DNA or RNA have been published, and large scale studies are underway. Whether this will be feasible and affordable remains to be seen. More information on diagnostic procedures as well as current and future methods for diagnostic or near diagnostic testing can be found in Chapter 9.

Trisomy 18 testing does not occur in isolation. It is, in fact, a reinterpretation of existing measurements that were justified for Down syndrome and open neural tube defect screening. Given the relatively low prevalence and poor survival after birth, any interpretation for trisomy 18 must be extremely efficient, resulting in a small number of procedures per case detected. Given this as background, there are several considerations for Down syndrome screening programs when reviewing and updating their protocols to identify trisomy 18 pregnancies. These include:

- Regardless of the Down syndrome test offered, if a trisomy 18 interpretation is to be made, the age-associated prior risks and fetal loss coefficients by gestational age should be updated to the most currently available information.
- Patient and provider materials should be reviewed to ensure that the natural history is correctly portrayed and variability in phenotype considered.

- If first trimester combined Down syndrome screening is offered, trisomy 18 interpretations can be provided, but updating NT parameters is critical for assigning reliable risks. Programs should also review their expected performance claims against those provided here.
- Available evidence suggests that a similar percentage of first trimester trisomy 13 pregnancies will be coincidentally detected by the trisomy 18 risk interpretation.
- There is insufficient information to recommend the routine use of other first trimester ultrasound markers (e.g., nasal bone, ductus venosus) for trisomy 18.
- If second trimester Down syndrome triple marker testing is offered, trisomy 18 interpretations can be provided using updated parameters. However, consideration should also be given to strategies that could utilize PAPP-A measurements from that sample.
- Routine use of second trimester ultrasound makers as part of routine testing for trisomy 18 does not appear useful. Modifying trisomy 18 risks using these ultrasound markers is also problematic, as it would reduce the high detection already present with excellent available serum/NT testing.
- If full integrated Down syndrome screening is offered, trisomy 18 interpretations could utilize first trimester PAPP-A and NT measurements, along with second trimester triple markers, using the updated parameter sets.
- If serum integrated Down syndrome screening is offered, trisomy 18 interpretations should utilize first trimester PAPP-A, along with second trimester triple markers using updated parameters.
- The performance of sequential testing for trisomy 18 can approach that for full integrated testing. However, such testing would likely occur only if the laboratory utilized sequential testing for Down syndrome.

The best practices for identifying trisomy 18 can be summarized in four distinct categories that can co-exist in clinical practice. For those women presenting prior to 14 weeks' gestation and who have access to reliable NT measurements, the full integrated test has the highest performance. However, the performance of a 'sequential' integrated test is essentially the same and has an advantage in identifying a high proportion of trisomy 18 fetuses in the first trimester rather than in the second trimester. Sequential testing involves setting a very high risk cut-off level in the first trimester to interpret the NT, PAPP-A measurements (with or without the hCG subunit measurement). The reason for this is that the risk is so high, that the additional information available in the second trimester (AFP, uE3 and hCG measurements) would be unlikely to reclassify this

pregnancy as being at low risk. Thus, action can be taken earlier in pregnancy in this small group. The vast majority of pregnancies that are not at this very high risk, continue with an integrated test, only receiving their risk after the second trimester testing has been completed. If NT is not available in this group of women presenting early for prenatal care, serum integrated testing is the next best option. For those women presenting later in pregnancy, the 'triple' test would be best practice, with an option for adding PAPP-A measurements as the fourth marker. Lastly, for those women who strongly favor a first trimester diagnosis over higher test performance, the first trimester combined test is appropriate.

The next four sections rely on flow charts to show how each of these tests perform in identifying trisomy 18 fetuses, among a general pregnancy population of 1,000,000 singleton pregnancies. The modeling shown here is sufficiently complete to allow for decision-making regarding protocol selection. It is not, however, a comprehensive screening model. For example, the model consists only of trisomy 18 and unaffected pregnancies; disregarding other pregnancy outcomes. There are two important implications of this. Some of the women who have positive results for trisomy 18 will also have positive results for Down syndrome. Thus, these models provide the detection rate for trisomy 18, not the marginal increase in trisomy 18 detection. As shown earlier, trisomy 18 test performance is extremely good, with high detection rates at very low false positive rates, usually below 0.3% (3 per 1000 false positives). At such low rates, the model will not agree well with rates found in practice. This is because other relatively rare outcomes (*e.g.*, fetal death, anencephaly) that the trisomy 18 algorithms preferentially identify can result in a noticeable increase in the false positive rate (*i.e.*, from 0.1% to 0.3%). However, most of these additional 'false positives' are not unaffected pregnancies. With these cautions in mind, the four testing protocols for trisomy 18 are presented below.

Sequential testing If NT measurements are available between 11 and 13 weeks' gestation, the sequential test would be considered the optimal strategy. The steps in sequential testing of 1,000,000 singleton pregnancies for trisomy 18 are shown in Figure 10-2. Since the test starts in the first trimester, the prevalence of trisomy 18 is relatively high (9.61 per 10,000 versus 2.69 per 10,000 at term). A reasonable first trimester cut-off is about 1:6 (equivalent to a term risk of 1:20). Among the unaffected pregnancies, the false positive rate is less than 0.1% leading to about 500 false positives. Among the trisomy 18 pregnancies, the 70% detection rate translates into 673 screen positive cases. Together, these 1,173 women could be offered first

trimester diagnostic testing, with an OAPR of about 1:1 (673:500). All of the remaining 998,769 pregnancies would continue on for second trimester testing. It would be preferable, if this group of women did not receive their first trimester risk result.

In the second trimester, the triple markers would be added to the risk computation and using a second trimester risk cut-off of about 1:100 (term risk of 1:300), the detection rate for the remaining affected pregnancies would be about 67%. The corresponding false positive rate would again be about 0.05%. The OAPR would be 1:2 and the OANR about 1:9,200. Overall, the total detection rate is 90%, at a 0.1% false positive rate. After accounting for fetal loss, there is the potential to reduce the birth prevalence from 2.69 to 0.27 per 10,000. This performance is very nearly the same as would be found for full integrated testing.

Serum integrated testing Such testing is indicated if the gestational age is prior to 14 weeks' gestation, but reliable NT measurements are not available (Figure 10-3). The second trimester prevalence is used, as that is the time when the risk will be reported. Using a second trimester risk cut-off level of 1:105 (equivalent to 1:300 at term) the detection and false positive rates are 85% and 0.16%, respectively. High risks will be assigned to 646 affected, and 1,599 unaffected pregnancies. The OAPR will be about 1:3, while the OANR is about 1:8,100. After accounting for fetal loss, there is the potential to reduce the birth prevalence from 2.69 to 0.43 per 10,000.

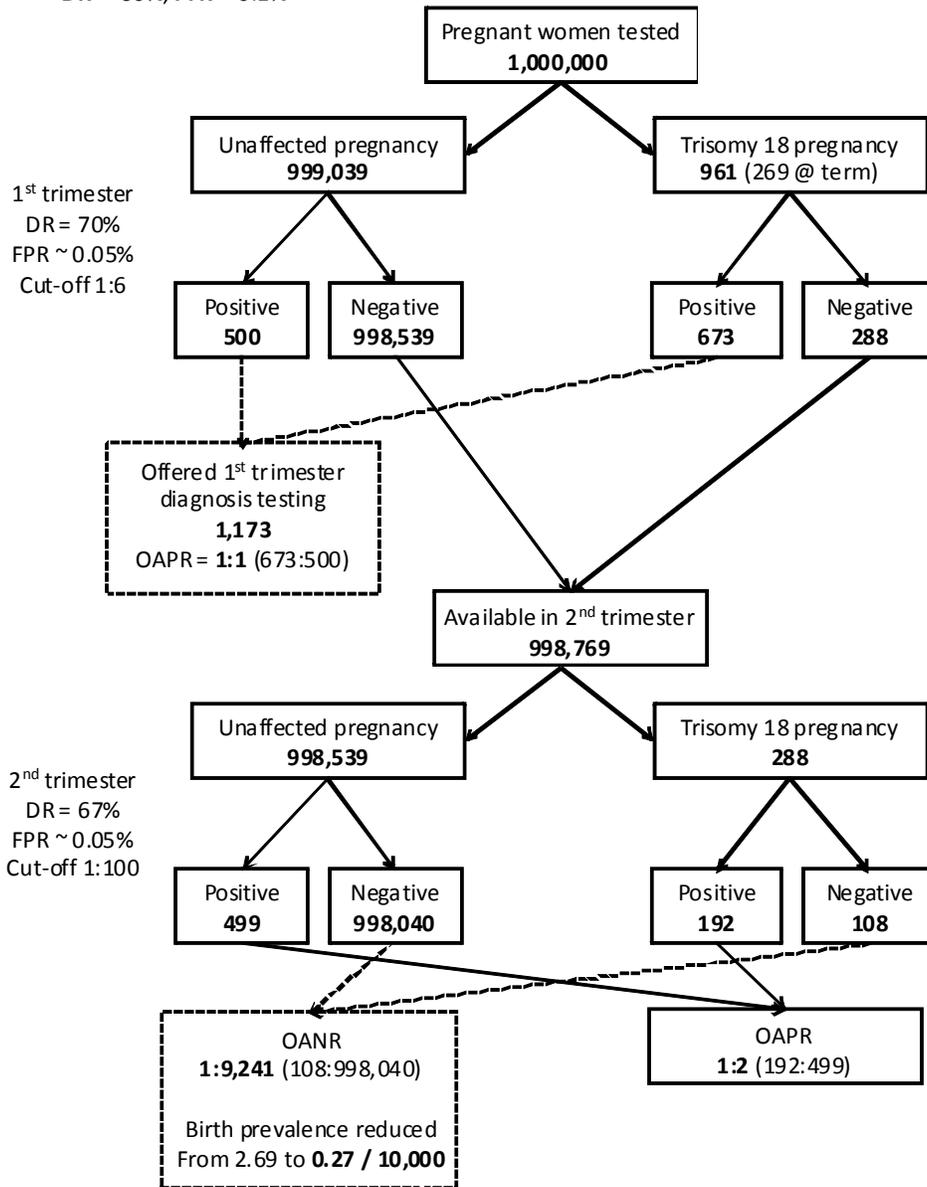
Second trimester testing Figure 10-4 shows the steps in second trimester testing. The prevalence of trisomy 18 at that time in pregnancy is less than the 961 found earlier for the first trimester, but the 769 is still considerably higher than the 269 expected at term. The most commonly used algorithm for trisomy 18 utilizes the existing 'triple test' markers (flowchart on the left of Figure 10-4). Using a second trimester risk cut-off level of 1:35 (equivalent to 1:100 at term) the detection and false positive rates are 73% and 0.12%, respectively. This leads to 561 trisomy 18 and 1,119 unaffected pregnancies being assigned to the high risk category. The OAPR is about 1:2 and the OANR is about 1:4,800. After accounting for fetal loss, there is the potential to reduce the birth prevalence from 2.69 to 0.73 per 10,000.

Figure 10-4 also shows an improved second trimester algorithm that requires an additional measurement of PAPP-A (flowchart on the right). At the same risk cut-off level, the detection rate improves to 81% and the false positive rate is slightly

reduced to 0.1%. This leads to 623 trisomy 18 pregnancies and 999 unaffected pregnancies assigned to the high risk category. The OAPR is about 2:3; the OANR is about 1:6,800. There is the potential to reduce the birth prevalence from 2.69 to 0.52 / 10,000. The performance would be similar, even if only a portion of the population were to have PAPP-A testing in a sequential protocol (*i.e.*, only perform PAPP-A testing on women with very high risks as assigned by the triple test).

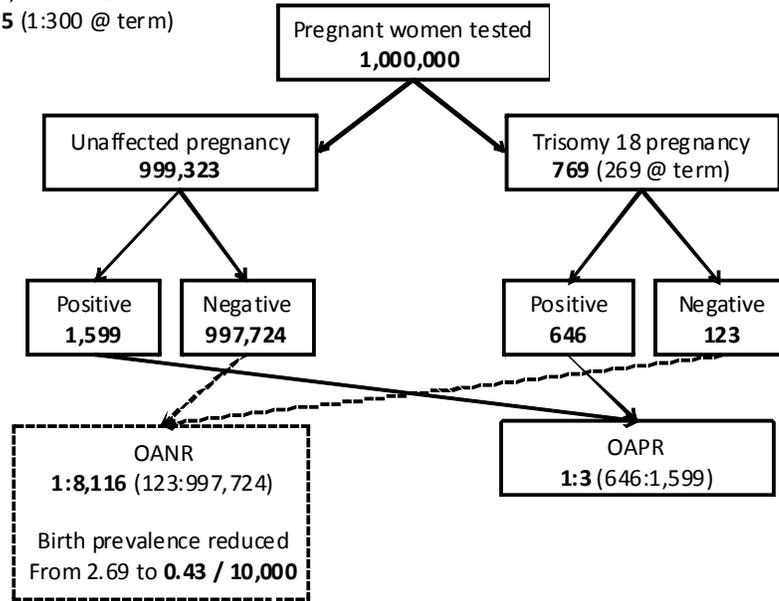
First trimester testing Figure 10-5 shows the steps in combined testing (NT, PAPP-A and free  $\beta$  hCG) in 1,000,000 singleton pregnancies. Since testing begins in the first trimester, the prevalence of trisomy 18 in this population is again 961 (9.61 per 10,000). Using a first trimester risk cut-off level of 1:29 (equivalent to 1:100 at term), the combined test has a detection rate of 86% and false positive rate of 0.2%. Thus, 826 of the affected pregnancies, along with 1,998 of the unaffected pregnancies will be assigned a risk of 1:29 or higher. In this group, the OAPR is about 1:2; the OANR is 1:6,800. After accounting for fetal loss, there is the potential to reduce the birth prevalence from 2.69 to 0.52 per 10,000.

**Sequential test**  
 DR = 90%, FPR = 0.1%



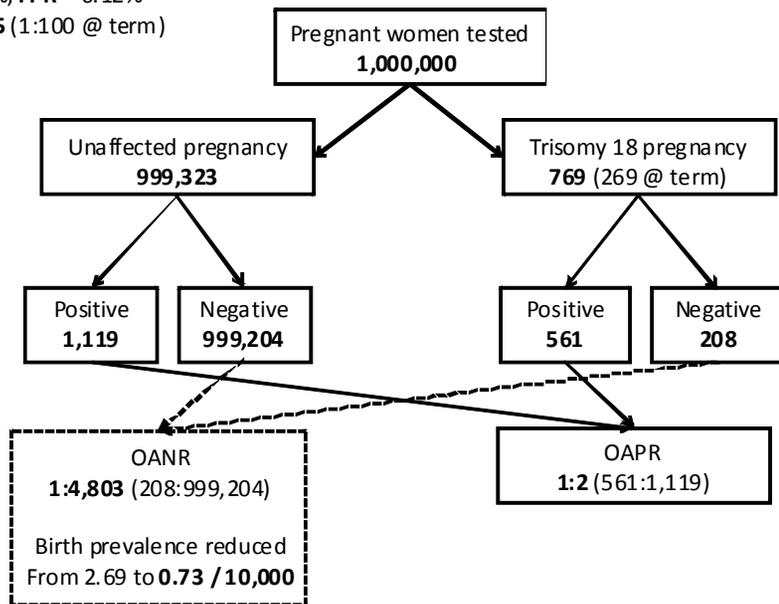
**Figure 10-2. Sequential testing for trisomy 18 in 1,000,000 singleton pregnancies in the general population.** The trimester-specific prevalence of trisomy 18, age associated risks and the test's detection and false positive rates are taken from summary data presented in this chapter.

**Serum Integrated test**  
 DR = 84%, FPR = 0.16%  
 Cut-off 1:105 (1:300 @ term)

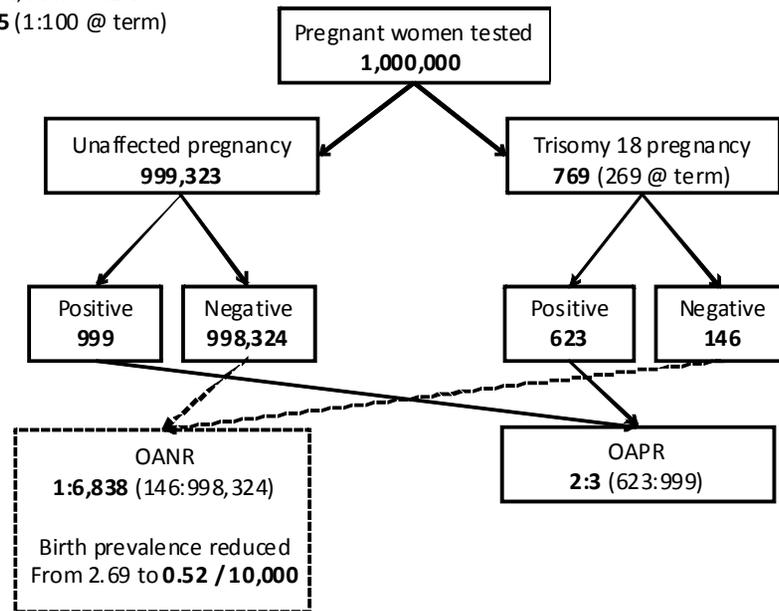


**Figure 10-3. Serum integrated testing for trisomy 18 in 1,000,000 singleton pregnancies in the general population.** The trimester-specific prevalence of trisomy 18, age associated risks and the test's detection and false positive rates are taken from summary data presented in this chapter.

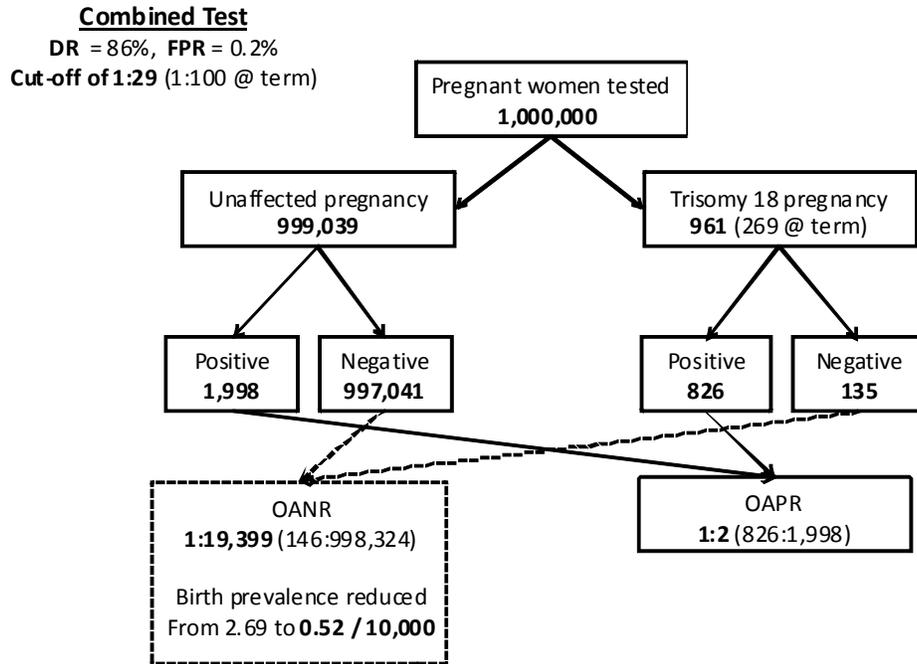
**Triple Test**  
 DR = 73%, FPR = 0.12%  
 Cut-off 1:35 (1:100 @ term)



**Quadruple test**  
 DR = 81%, FPR = 0.1%  
 Cut-off 1:35 (1:100 @ term)



**Figure 10-4. Second trimester testing for trisomy 18 in 1,000,000 singleton pregnancies in the general population.** The left hand flow diagram shows performance of the ‘triple test’ composed of AFP, uE3 and hCG measurements. The right hand flow diagram shows the changes in performance if a second trimester PAPP-A measurement were added to create the ‘quadruple test’. The trimester-specific prevalence of trisomy 18, age associated risks and the test’s detection and false positive rates are taken from summary data presented in this chapter.



**Figure 10-5. First trimester ‘combined’ testing for trisomy 18 in 1,000,000 singleton pregnancies in the general population.** The trimester-specific prevalence of trisomy 18, age associated risks and the test’s detection and false positive rates are taken from summary data presented in this chapter.

Since trisomy 18 testing is a re-interpretation of existing markers for Down syndrome, the timing and availability of marker results and type of testing offered (e.g., sequential, combined) will be determined by the Down syndrome screening test(s) offered. The application of results in this thesis and considerations of the above suggested recommendations and best practices should make it possible to identify nearly all trisomy 18 pregnancies for a very low false positive rate.