

COMBINED PRE-ECLAMPSIA
SCREENING WITH
THE PIGF 1-2-3™ ASSAY

HAND BOOK

Brochure not for distribution in the USA


PerkinElmer[®]
For the Better

CONTENTS

Foreword	5
Section 1 - Introduction.....	6
1. What is pre-eclampsia?.....	6
2. The global burden of pre-eclampsia.....	6
Pre-eclampsia is more common than aneuploidies	7
3. The cost of pre-eclampsia	8
4. Early (<34 weeks), preterm (<37 weeks) and term pre-eclampsia (≥ 37weeks).....	9
Is pre-eclampsia a spectrum disorder?.....	9
5. A new era in pre-eclampsia care – ASPRE	10
Why is the ASPRE study so important?	10
Implications of the ASPRE study	11
Cost-effectiveness of screening	12
6. The principle of combined pre-eclampsia screening	13
7. Pre-eclampsia screening markers.....	15
Biochemical markers.....	15
Biophysical markers	15
Section 2 - Clinical implementation	16
8. When should combined pre-eclampsia screening be performed?	16
9. Who should the pre-eclampsia screening be offered to?	16
10. Combined pre-eclampsia screening	17
Step 1. Interview to establish medical and obstetric history factors contributing to background risk	17
Step 2. Blood test to measure PIGF and, possibly, PAPP-A.....	18
Step 3. Blood pressure – MAP	20
Step 4. Ultrasound – uterine artery pulsatility index (UTPI)	24
Step 5. Automatic risk assessment.....	25
11. The right combination of markers	29
Section 3 - Management of screen positive pregnancies	30
12. Aspirin treatment according to ASPRE	30
Frequently asked questions on aspirin therapy	30
Section 4 – On informing parents about pre-eclampsia	33
13. What parents need to know.....	33
Pre-eclampsia screening and maternal anxiety.....	33
Pre-screening information	34
Post-screening information	36
Appendix	37
References	38

Foreword

Nowadays, pregnant women expect doctors to offer effective prenatal care based on the latest research evidence, screening and preventive solutions. They want to receive the best clinical care possible throughout the pregnancy.

With regard to pre-eclampsia, we are stepping into a new era in terms of how the disease is predicted and managed. The ASPRE study has now given definitive evidence that the best way to reduce the rate of preterm pre-eclampsia (with delivery before 37 weeks' gestation) is through early prediction and prevention by low-dose aspirin. A significant 62% reduction in the rate of preterm pre-eclampsia amongst women who would otherwise develop the condition is great news for women at risk and their families.

This booklet, which I have reviewed, is an ASPRE-inspired, practical introduction to combined pre-eclampsia screening and management. My personal post-ASPRE goal is to make this knowledge accessible to all health care professionals and pregnant women.



And my message: Yes, it is time for clinical implementation.

Prof. Liona Poon

*The Chinese University of Hong Kong
The Fetal Medicine Foundation*

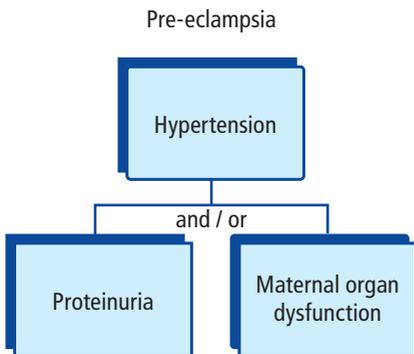
Liona Poon is devoted to improving maternal and fetal health. She has a prolific research output including over 130 peer-reviewed publications in high impact international journals. In the past 10 years, she has focused her research on establishing a programme for effective early prediction and prevention of pre-eclampsia, which is a major cause of maternal and perinatal morbidity and mortality. With her success in the conduct of the pan-European multicenter randomized controlled trial of Aspirin vs placebo in the prevention of preterm pre-eclampsia (ASPREE), her goal in the next 10 years is to improve obstetric care worldwide, through clinical research and education.

This booklet represents an attempt to provide a summary of current opinions on pre-eclampsia screening and prevention. All information presented in this booklet is for informational purposes only and is not intended as medical advice. PerkinElmer does not endorse or make recommendations with respect to research, medication, or treatments. For country specific recommendations, please consult your local health care professionals.

Section 1 – Introduction

1. What is pre-eclampsia?

Pre-eclampsia is a complication of pregnancy marked by high blood pressure and presence of protein in the urine (proteinuria). In the absence of proteinuria, the finding of maternal organ dysfunction is sufficient to make the diagnosis of pre-eclampsia.



Pre-eclampsia develops as a result of abnormal blood flow to and within the placenta, which in turn can cause growth-restriction or preterm birth of the child. When left untreated, pre-eclampsia can lead to eclampsia, a serious condition that can endanger the mother's life.^[1]

Figure 1. Pre-eclampsia is now diagnosed by high blood pressure and presence of protein in the urine. In the absence of proteinuria, the finding of maternal organ dysfunction is sufficient to make the diagnosis of pre-eclampsia

2. The global burden of pre-eclampsia

Pre-eclampsia affects 2–8% of pregnancies. The incidence is increasing with the global increase in maternal age, obesity and the use of assisted reproductive techniques. It also follows the rising incidence of diabetes, hypertension, and renal disease – all are known co-morbidities that predispose sufferers to pre-eclampsia during pregnancy.

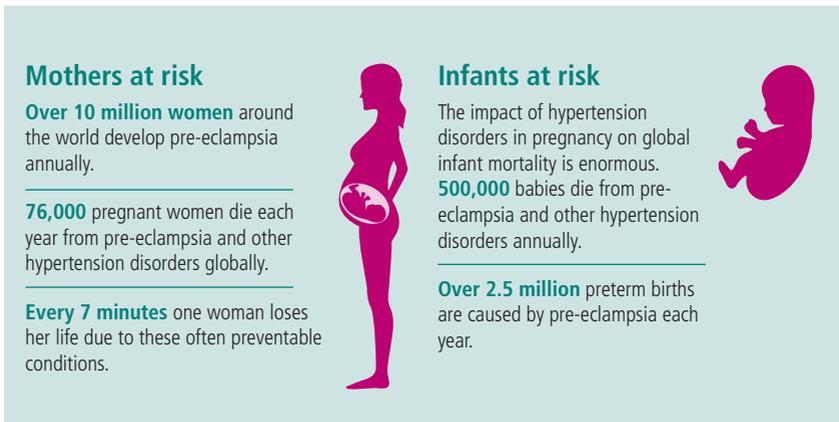


Figure 2. The burden of pre-eclampsia. The figures are estimates.^[4]

In one third of the cases the condition leads to delivery at <37 weeks' gestation (preterm pre-eclampsia) and in two thirds delivery occurs at ≥ 37 weeks (term pre-eclampsia).^[2,3]

There is evidence that pre-eclampsia also leads to long term health problems. Women who develop pre-eclampsia have twice the risk of cardiovascular disease (CVD) at some stage in their life. It is not known whether pre-eclampsia causes vascular damage that ultimately leads to CVD or whether women prone to CVD are stressed by pregnancy to develop pre-eclampsia.^[5,6]

Children born to pre-eclamptic mothers have twice the risk of cerebral palsy; this increased risk being mediated through premature birth, growth-restriction or both. Offspring exposed to pre-eclampsia also have higher blood pressure and body mass index, compared to those born after normal pregnancy, and have increased risk of CVD and diabetes in adult life.^[5,6]

Pre-eclampsia is more common than aneuploidies

For the past 20 years, the focus of prenatal screening programs has been for fetal Down syndrome (trisomy 21). The prevalence of pre-eclampsia and related conditions (fetal growth-restriction and preterm birth) is much higher than that of Down syndrome. Furthermore, unlike Down syndrome, pre-eclampsia is a major cause of maternal and perinatal morbidity and mortality. Preventing pre-eclampsia would bring substantial improvements to maternal and perinatal health.^[7,8,9,10]

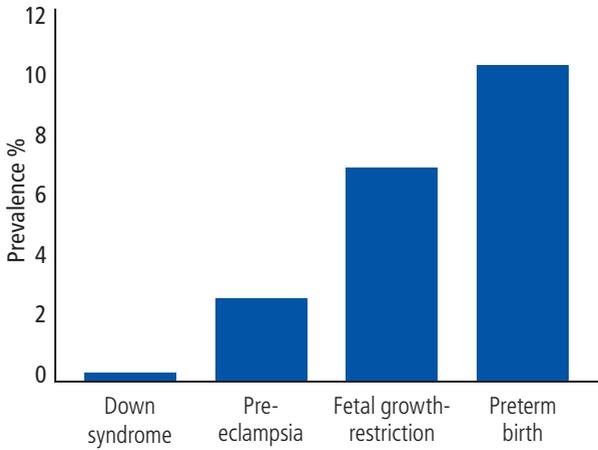


Figure 3. A comparison between the prevalence of Down syndrome vs. pre-eclampsia and conditions associated with pre-eclampsia such as fetal growth-restriction and preterm birth.

In fact, pre-eclampsia is more common than all aneuploidies combined, and both the mother and the baby are affected. Avoiding pre-eclampsia would bring substantial improvements to maternal and fetal health.

3. The cost of pre-eclampsia

The health care cost burden of pre-eclampsia is extensive. In addition to the severity of the disease, the degree of prematurity is extremely important. About 80% of all pre-eclampsia related costs are caused by preterm pre-eclampsia (with

Table 1. Short-term costs of pre-eclampsia to the United States health care system.^[11]

Estimated unit and total health care cost for pre-eclampsia patients in the United States, by gestational age at birth (2012) using California Office of Statewide Health Planning and Development and commercial claims data					
Costs	<28 wks (2604)	28-33 wks (23,624)	34-36 wks (61,856)	≥7 wks or longer (87, 596)	All (156, 680)
Maternal cost per birth	\$29,131	\$24,063	\$19,692	\$17,021	\$19,075
Infant cost per birth	\$282,570	\$59,803	\$11,112	\$6013	\$21,847
Combined cost per birth	\$311,701	\$83,866	\$30,804	\$23,035	\$40,922
Total health care cost	\$1.2 billion	\$2.0 billion	\$1.3 billion	\$2.0 billion	\$6.4 billion
Total cost because of infant cost, %	91%	71%	36%	26%	

delivery at ≤ 37 weeks). Much of the total cost burden is associated with care of the preterm neonate, which is highly dependent on the gestational age at birth. The cost burden of pre-eclampsia during the first 12 months after birth per infant is estimated to range from \$150,000 at 26 weeks gestational age to \$1311 at 36 weeks gestational age. ^[11]

4. Early (<34 weeks), preterm (<37 weeks) and term pre-eclampsia (≥ 37 weeks)

Is pre-eclampsia a spectrum disorder?

Early and preterm pre-eclampsia – placental origin

While the direct cause of pre-eclampsia is unknown, researchers agree that if pre-eclampsia requires delivery before 37 weeks, there is a high chance that poor placentation is the underlying cause of the disease.

Early onset pre-eclampsia is associated with preterm birth and fetal growth-restriction, with prematurity accounting for most pre-eclampsia-related healthcare costs. If HELLP syndrome or eclampsia occurs, intensive care is probable. ^[12]

The ASPRE study and meta-analyses have shown that aspirin treatment is highly effective in the prevention of early and preterm pre-eclampsia. ^[12,13]

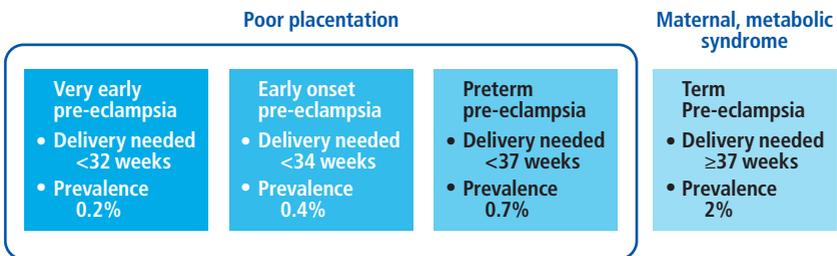


Figure 4. Poor placentation is associated with very early, early and preterm pre-eclampsia.

Term pre-eclampsia – Maternal and cardiac origin

New evidence suggests that in term pre-eclampsia (with delivery after 37 weeks) the resulting condition is more closely related to cardiac and metabolic dysfunction in the mother rather than poor placentation. In fact, term pre-eclampsia has been hypothesized to be a completely different pregnancy complication to early and preterm pre-eclampsia. ^[14]

Is pre-eclampsia a spectrum disorder?

The alternative view is that pre-eclampsia is a spectrum disorder in which the severity of the condition is reflected in the gestational age at clinical presentation and delivery for the condition. ^[15,16]

5. A new era in pre-eclampsia care – ASPRE

The ASPRE team has successfully shown that the use of aspirin treatment for screen positive pregnant women dramatically reduces the risk of preterm pre-eclampsia if administration of the optimal dosage of aspirin is started during the first trimester of pregnancy. ^[13]

PerkinElmer is proud to have been chosen as a partner for this groundbreaking study in which our high sensitivity PIGF 1-2-3™ assay was used.



Figure 5. Aspirin treatment reduces the prevalence of preterm pre-eclampsia. ^[13]

Why was the ASPRE study so important?

ASPRE was the biggest prospective, randomized, placebo controlled trial ever on the prophylactic use of aspirin in women at increased risk of pre-eclampsia. The study was conducted in order to bring final, conclusive evidence about the efficacy of aspirin in prevention of preterm pre-eclampsia in women at high risk of the disease when high risk is defined using the first trimester combined screening method. ^[13,17]

Briefly described, 27,000 singleton pregnancies were screened using the combined screening approach at 11-13⁺⁶ weeks' gestation. Screen positive women (cases in which the risk for preterm pre-eclampsia was >1 in 100) were randomly allocated to receive aspirin (150 mg/day) or placebo from 11-13⁺⁶ weeks' gestation until 36 weeks. Participants were recommended to take the tablet at night, rather than during the day, because there is evidence of increased efficacy if taken at bedtime. Participants, researchers and managing clinicians were blinded to the treatment. ^[18]

The ASPRE results showed that use of aspirin was associated with a significant 62% reduction in the incidence of preterm pre-eclampsia (<37 weeks GA) and an 82% reduction in the incidence of early onset pre-eclampsia (<34 weeks GA). ^[13]

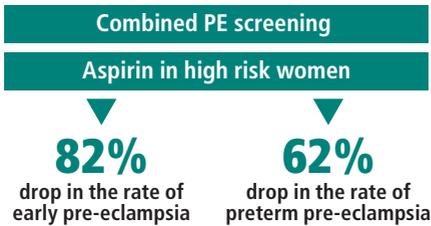


Figure 6. ASPRE results showing how aspirin treatment following combined pre-eclampsia screening reduced incidences of early pre-eclampsia (<34 weeks) and preterm pre-eclampsia (<37 weeks).

Implications of the ASPRE study

The ASPRE study has given definitive evidence that the best way to reduce the risk of preterm pre-eclampsia is through early combined screening and intervention. ^[13,17]

Secondary analysis of the ASPRE data further showed that the beneficial effect of aspirin depended on compliance. For women conscientiously taking more than 90% of the daily tablets, the reduction in the incidence of preterm pre-eclampsia (<37 weeks) was as high as 75%, compared to 62% (see figure 6) when any level of compliance was considered.

Further, the beneficial effect of aspirin in reducing the risk of preterm pre-eclampsia may not apply to women with chronic hypertension though the number of cases included in the trial was small. On the other hand, screen positive women but without chronic hypertension, who took more than 90% of the daily

tablets, could experience a reduction in the rate of preterm pre-eclampsia as high as 95%.^[17]

Recent analysis of the ASPRE data revealed that administration of 150mg aspirin at bedtime started from 12 weeks reduced the length of stay in the neonatal intensive care unit by 68%, which was highly significant. This could be, mainly attributed to a decrease of births at <32 weeks' gestation.^[19]

Outcome measure	Aspirin	Placebo	
Length of stay in NICU (d)			Diff in means (95% CI)
Study population: admission	N=49	N=54	
Mean (SD)	11.1 (23.4)	31.4 (53.0)	20.3 (7.0-38.6)
Study population: all cases in the trial	N=798	N=822	
Mean (SD)	0.66 (6.3)	2.06 (15.5)	1.40 (0.45-2.81)
No. of babies in NICU			OR (95% CI)
Study population: livebirths	N=777	N=794	
Number by GA at birth			
Any, n (%)	48 (6.2)	54 (6.8)	0.94 (0.63-1.42)
PE	2 (0.3)	18 (2.3)	0.11 (0.02-0.50)
No PE	46 (5.9)	36 (4.5)	1.38 (0.88-2.15)
<32w, n (%)	9 (1.2)	23 (2.9)	0.42 (0.19-0.93)
PE	0	7 (0.9)	0.00 (0.00-0.56)
No PE	9 (1.2)	16 (2.0)	0.59 (0.26-1.36)
Length of stay (d)	531	1696	

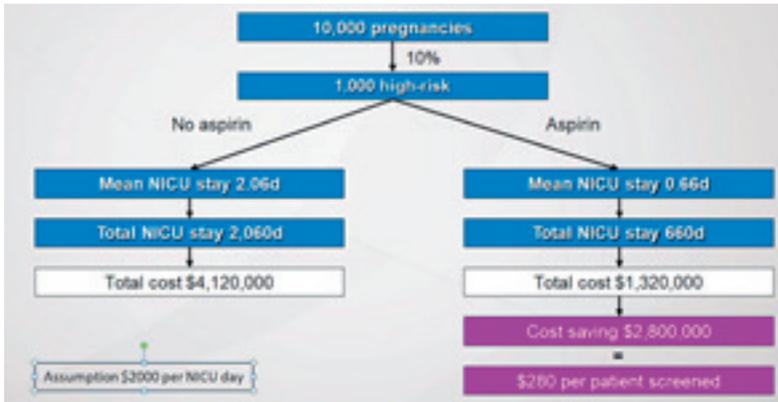
Data courtesy of Liona Poon

Figure 7. ASPRE results showing how aspirin treatment following combined screening reduced the length of stay in the neonatal intensive care unit. NICU = neonatal intensive care unit, d = day, CI = confidence interval, SD = standard deviation, OR = odds ratio, GA = gestational age, PE = pre-eclampsia.

This finding has implications to both short and long term healthcare costs as well as infant survival and disability.

Cost-effectiveness of screening

As an illustration, if we assess the impact of screening on a population of 10,000 women, and on the basis of the ASPRE screening test consisting of maternal history, uterine artery pulsatility index (UTPI), mean arterial blood pressure (MAP) and placental growth factor (PIGF), identified a 10% high risk group



Data courtesy of Liona Poon

Figure 8. Illustration of cost savings generated through the reduction in the length of stay in the neonatal care unit in a population of 10,000 women

with a 75% DR of preterm PE, then, we would expect to see an average reduction of 1.4 days per baby in time spent in NICU in high risk women treated with aspirin prophylaxis.^[19]

If we assume a cost of US\$2000 per day in NICU, this equates to a cost saving of \$2.8m per 10,000 women screened. On this basis, a cost per screen of \$280, would be cost neutral.^[19]

For institutions, where first trimester aneuploidy screening is already established, the additional cost of first trimester PE screening is likely to be well within this estimated amount. For those institutions, hospitals, diagnostic units and health care systems, without established first trimester ultrasound, it is still anticipated that at worst it would be cost neutral and is still anticipated to create cost savings.

6. The principle of combined pre-eclampsia screening

Combined pre-eclampsia screening, developed by the Fetal Medicine Foundation, allows estimation of individual, patient specific risk for developing pre-eclampsia later in pregnancy.^[15,16,20]

The approach to combined pre-eclampsia screening is similar to combined Down syndrome screening, where the prior risk obtained from maternal characteristics and medical history is combined with the results of biophysical and biochemical measurements (i.e. a blood test). However, the combined pre-eclampsia screening has added a new element with the use of a survival time model. This allows estimation of risk for pre-eclampsia requiring delivery before a specified gestational age. Theoretically, it is hypothesized that all women would develop pre-eclampsia if pregnancy were to continue indefinitely. There is a competition between delivery before or after the development of pre-eclampsia. [15,16]

The performance of the combined pre-eclampsia screening has been shown to be superior in identifying women at high risk of pre-eclampsia compared to the traditional approaches referenced in national guidelines. [20]

These traditional approaches are based on maternal characteristics along with medical, obstetric and family history. Although the information is easy to obtain, pre-eclampsia screening that is solely based on these factors is ineffective. Either the detection rate (DR) is poor or the screen positive rate is unacceptably high. [20,21]

Superior performance, especially for preterm (<37 weeks), early (<34 weeks) and very early pre-eclampsia (<32 weeks), is achieved with the combined screening method. [20,21]

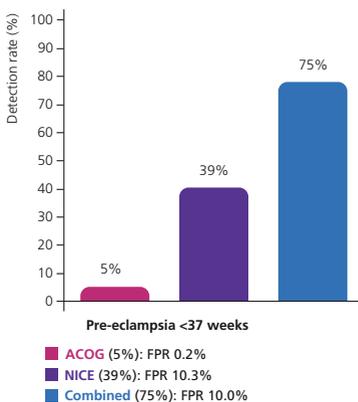


Figure 9. Detection rates (DR) with associated false positive rates (FPR) obtained using ACOG (American College of Obstetricians and Gynecologists) and NICE (UK National Institute for Health and Care Excellence) guidelines compared with that achieved by using combined pre-eclampsia screening. [20,22,23]

7. Pre-eclampsia screening markers

Biochemical markers

Biochemical markers that reflect placental function, such as placental growth factor (PIGF) and pregnancy associated plasma protein-A (PAPP-A), are significantly reduced in the first trimester, and throughout the pregnancy, in patients that will later present with preterm pre-eclampsia with delivery <37 weeks' gestation. Of these two markers PIGF is a better pre-eclampsia screening marker than PAPP-A (i.e. it has higher sensitivity). ^[24,25]

Biophysical markers

Mean arterial blood pressure (MAP) and the mean uterine artery pulsatility index (UTPI) between 11 weeks and 13+6 weeks' gestation are higher in women that will later develop pre-eclampsia compared to unaffected pregnancies, and are particularly raised in those women who develop the early form of the disease. ^[26,27]

PIGF ↓
PAPP-A ↓
MAP ↑
UTPI ↑

Figure 10. In early pregnancy values for biochemical markers are reduced and values for biophysical markers are elevated, indicating increased risk of pre-eclampsia.

Section 2 – Clinical implementation

8. When should combined pre-eclampsia screening be performed?

The optimal time for the combined screening is at 11–13 weeks' gestation. This is because aspirin treatment is only effective in improving the placentation and reducing the risk of preterm pre-eclampsia if started early.^[12,28,29]

Whilst it is possible to utilize a similar pre-eclampsia screening protocol later in the second and third trimesters, this is for planning surveillance and management only. This is because aspirin prophylaxis is not effective when started after 16 weeks' gestation. Timing and content of subsequent visits can be planned according to the assigned risk status. One can be alerted to the changes in the mother's or baby's condition and signs and symptoms of pre-eclampsia. This would potentially minimize adverse perinatal events for those that develop pre-eclampsia and allow planning of the appropriate time and place for delivery.^[28]

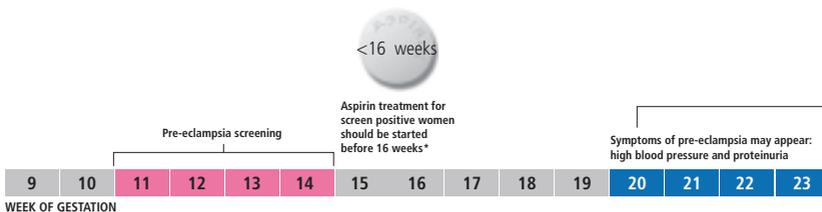


Figure 11. The optimal time window for combined pre-eclampsia screening.^[12,26,27]

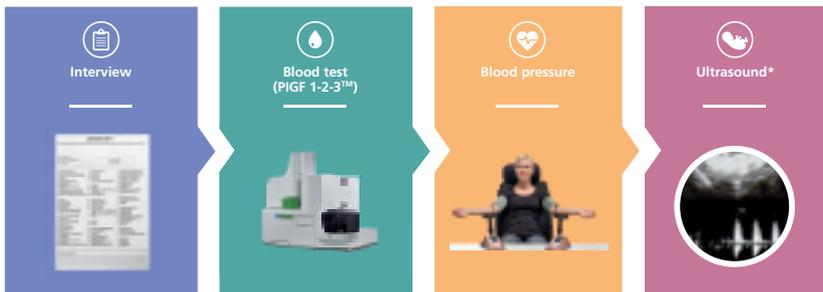
9. Who should the pre-eclampsia screening be offered to?

All pregnant women should be assessed early in their pregnancy and, as part of this assessment, preventing the subsequent development of pre-eclampsia should be regarded as a major objective. All women should have access to screening, even if there are no maternal risk factors or history of pre-eclampsia.^[30,31]

Being able to predict and prevent preterm pre-eclampsia early in the pregnancy is hugely beneficial, and such objectives are not achievable with a conventional wait-and-see approach to pre-eclampsia management.^[30]

10. Combined pre-eclampsia screening

The combined pre-eclampsia screening program is made up of four simple steps that require short training and minimal additional investment in equipment.



- 1. Record medical history, measure weight and height.**
- 2. Take blood sample for measuring PIGF 1-2-3™ (+/- PAPP-A)**
- 3. Measure blood pressure 2 times from both arms simultaneously with validated automated BP devices.**
- 4. Measure uterine artery Doppler pulsatility index by transabdominal ultrasound (FMF accreditation is necessary)**

Figure 12. The four steps involved in combined pre-eclampsia screening.

Step 1. Interview to establish medical and obstetric history contributing to background risk

Certain maternal characteristics and factors are known to increase the risk of pre-eclampsia. As part of combined pre-eclampsia screening, these risk factors need to be ascertained and documented by interviewing the pregnant woman. A background risk, obtained on the basis of these maternal characteristics and factors, will be subsequently adjusted according to the results of the blood test and biophysical markers.^[16,28]

Factors that increase the risk of pre-eclampsia

- Advancing maternal age
- Increasing weight
- Afro-Caribbean and South Asian racial origin
- Chronic hypertension
- Diabetes mellitus
- Systemic lupus erythematosus
- Antiphospholipid syndrome
- Conception by in vitro fertilization
- Family history or personal history of pre-eclampsia
- First pregnancy
- Multiple pregnancy

Factors that reduce the risk of pre-eclampsia

- The mother being tall rather than short
- Previous pregnancy without pre-eclampsia

Step 2. Blood test to measure PIGF

Placental Growth Factor (PIGF)

PIGF is mainly synthesized by the placenta. Women with an elevated risk of pre-eclampsia show a lower maternal serum level of this placental protein. This decrease is thought to be the consequence of placental hypoxia.^[28]

PIGF has been shown to be the most discriminating biochemical marker for pre-eclampsia.

PerkinElmer's high-sensitivity PIGF 1-2-3™ assay can be utilized for the measurement of free-PIGF (isoform PIGF-1) in the first trimester.^[32]

The same blood sample is used for the measurement of biochemical markers for both preeclampsia screening and aneuploidy screening using the same instrument. No additional blood sample is required, so the same sample can be used to screen for both pre-eclampsia and aneuploidy.^[32]

PAPP-A

Several publications have shown that first-trimester PAPP-A levels are also reduced in women who subsequently develop pre-eclampsia as compared with those who do not.^[33-36] Studies have shown PlGF to be a significantly stronger marker for pre-eclampsia than PAPP-A.^[36,37] In the combination of maternal history, MAP, UTPI and PlGF, addition of PAPP-A has not shown to provide additional improvement to screening performance. However, in the absence of PlGF, PAPP-A provides a small increase in the screening performance in combination with maternal history and MAP.^[38] In addition, if PAPP-A is measured for Down syndrome, it is beneficial to utilize the result also in the risk calculation for pre-eclampsia.

Sample collection and handling

The blood sample is collected by routine venipuncture, and allowed to clot. Serum is separated by centrifugation, taking care to follow the instructions of the sample tube manufacturer when selecting the clotting and centrifugation times. After centrifugation, the serum is immediately transferred to a new (plain) tube. This is especially important when using serum separator tubes (SST) with gel plugs.

PlGF is stable in maternal serum for 2 days at room temperature (+19– +25° C) when a 5% change in the PlGF concentration is allowed. If the separated serum sample cannot be measured on the same day of blood draw, the sample should be stored at +4°C for up to 30 days and at -20°C for longer periods.

To ensure the homogeneity of the sample prior to analysis, it is advised to gently invert the sample tubes several times before performing the analysis. On the following page we provide detailed advice on the handling of both fresh and stored samples prior to analysis.

Pre-analysis sample handling – how to ensure that the PIGF molecule is evenly distributed

- Fresh serum samples.
- Mix the serum tube no less than 5 times by inverting.

Refrigerated serum samples (+4°C)

- Allow the refrigerated serum sample to reach room temperature (19–25 °C). Note that this usually takes at least 30 min.
- Mix the serum tube by inverting no less than 10 times.

Frozen serum samples (-20°C)

- Allow the frozen samples to thaw and reach room temperature (19–25 °C). Note that thawing to room temperature can take a considerable time, especially if several frozen tubes are in a rack together.
- Mix the serum tube by inverting no less than 10 times.

Please note the following

- Mixing the samples using a vortex or tilting table is not recommended; these methods are less efficient than mixing by inverting.
- After the sample has been mixed by inverting it should not be spun or centrifuged.
- The PIGF assay should be run from the serum tube within the same day.
- Make sure that all assay reagents and solid reagents have reached room temperature (19–25 °C). Note that the liquid assay reagents need at least an hour and solid reagents at least 30 minutes for this.

More information on sample handling and stability can also be found in the PerkiElmer PIGF kit insert, in the Specimen Collection and Handling section. See also references ^[32] and ^[39].

Step 3. Blood pressure – MAP

In the prediction of pre-eclampsia, the calculated MAP is more useful than systolic and diastolic blood pressures. The MAP is defined as the average arterial pressure during a single cardiac cycle, and it is calculated from the systolic and diastolic readings using the equation:

MAP = (systolic blood pressure - diastolic blood pressure)/3 + diastolic blood pressure

The risk calculator program will perform this calculation, using the two recordings you have made for each arm, a total of 4 measurements. The program will then calculate the MAP multiple of the median (MoM), adjusted for the mother's weight, height, race, smoking, chronic hypertension, diabetes, family history of pre-eclampsia and previous history of pre-eclampsia. [26,28,38]

When using MAP as one of the pre-eclampsia screening markers, it is important to follow the standardized measurement protocol described below. This is the only way to secure the necessary level of accuracy. [26]

In addition, only automated blood pressure monitors that are validated for use in pregnancy and pre-eclampsia should be used (see below and Table 2). Mercury sphygmomanometers should not be used due to safety issues as well as concerns about their clinical performance.

How to position the patient correctly

The woman should be comfortably seated with her back supported and her legs uncrossed. Crossing of the legs may raise the MAP. The arms should be extended out, supported at heart level, and they should be free of clothing. If the arm is below the level of the heart, the MAP is over-estimated, and if it is above the heart, the MAP is under-estimated. If the arm is held up by the patient without support, the MAP will also be over-estimated. [26,28]

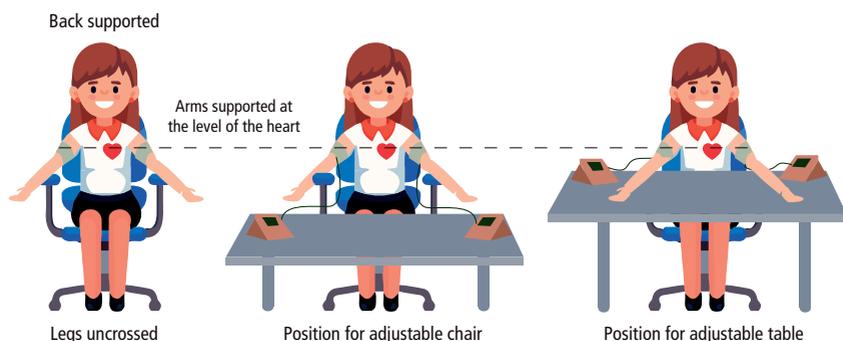


Figure 13. Positioning of the patient for MAP measurement

How to place the cuffs

There are small, medium and large cuff sizes, and you must choose the correct size. Using a cuff of the wrong size can cause inaccurate blood pressure readings. Too small a cuff may lead to an artificially high reading, while too large a cuff may cause the reading to be too low.

To choose the right cuff size, you might need to measure the mid arm circumference of the patient's arm. The inflatable part of the blood pressure cuff should cover about 80% of the mid arm circumference of the upper arm. The cuff should cover two-thirds of the distance from the patient's elbow to her shoulder.

Take care when positioning the cuffs. Using two blood pressure monitors, place the cuffs' sensors directly over the brachial pulse on the left and the right arms. The arms should be free of clothing. Cuff pressure will vary in synchrony with the brachial artery, therefore direct contact with the skin is necessary. Blood pressure measurement with automated devices is based on the oscillometric method. They use an electronic pressure sensor cuff. The values for systolic and diastolic pressure are computed using a proprietary algorithm.^[26,28,40]

How to perform MAP measurements

The following instructions should be followed.

- Take the measurements in both arms simultaneously.
- Let the patient rest for five minutes before measurements to minimize the chance of *white coat hypertension* – this is the phenomenon by which patients' blood pressure level rises as a result of their being in a clinical setting.
- After the first measurements, wait for 1 minute from the moment of cuff deflation, and then take a repeat set of measurements (again from both arms).
- Neither observer nor patient should talk during the measurement.

Evidence shows that the performance of screening for pre-eclampsia by MAP is best when simultaneous readings from both arms are considered.

Which blood pressure monitors can be used?

Blood pressure should be taken using validated automated monitors, which are calibrated at regular intervals. Validation specifically for pregnancy and pre-eclampsia is important because many automated monitors recommended for the

adult population systematically lead to underestimated values in pregnancy and pre-eclampsia. This is because hemodynamic or cardiovascular changes associated with pregnancy and pre-eclampsia (physiologic changes in pregnancy, decreased arterial compliance, changes in cardiac output and interstitial edema) delay the transmission of oscillations leading to unreliable recordings. ^[28,40]

Only certain monitors have the required technology and/or algorithms to allow them to be used with both pre-eclamptic and non-pre-eclamptic pregnant women.

Models validated for use for pregnancy and pre-eclampsia measurements are listed in Table 2. Models not listed can be used if they have been validated for pregnancy and pre-eclampsia or declared identical to a validated model. Please refer to the manufacturers' website for further details.

The lifetime of an automated device depends on the capacitive sensor. This can vary from 10,000 to 30,000 measurements.

Table 2. Automated blood pressure monitors reduce certain operator bias such as terminal digit preference. Below are some examples of models validated for use in pregnancy and pre-eclampsia measurements. ^[41,42]

Manufacturer	Model
Microlife	Watch BP Home
Microlife	BPA200
Microlife	3AS1-2
Microlife	BP 3BTO-A
Omron	MIT-Elite
Omron	MIT
Omron	Hem 705CP
Welch Allyn	Spot Vital Signs
Dinamal	ProCare 400

Step 4. Ultrasound – uterine artery pulsatility index (UTPI)

The UTPI can be measured between 11–13+6 weeks via a transabdominal ultrasound examination. [27,28]

The Fetal Medicine Foundation provides a detailed protocol for the UTPI measurement. It also awards certificates of competence to Health Care Professionals who have been trained to measure the UTPI.

Requirements for certification

The requirements for obtaining the Fetal Medicine Foundation certificate of competence in pre-eclampsia screening are:

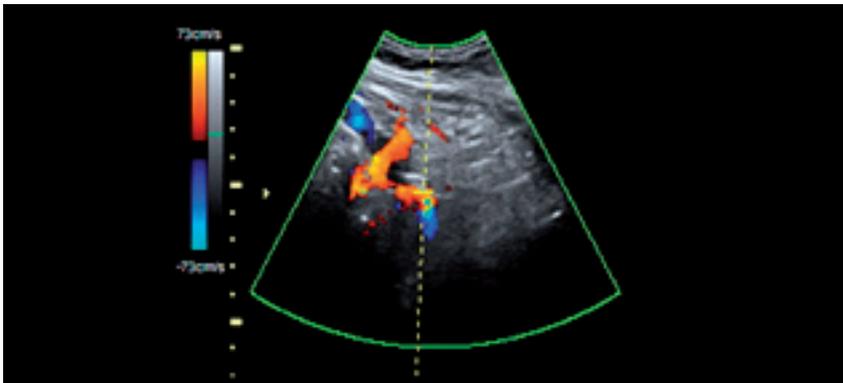
- Participation in a free online course on pre-eclampsia screening.
- Submission of 3 images demonstrating color flow mapping and waveforms of the uterine artery at 11–13+6 weeks.

For more information, please visit the Fetal Medicine Foundation website (www.fetalmedicine.org)

How to perform UTPI in the first trimester

- Identify uterine arteries.

Transabdominal UTPI



- Fix the probe in the midline.
- Obtain a sagittal section of the uterus and identify the cervical canal and internal cervical os.
- Use color Doppler.
- Tilt the transducer from side to side to identify the uterine arteries at the level of the internal cervical os.

After identification of each uterine artery, pulsed wave Doppler should be used with the sampling gate set at 2 mm to cover the whole vessel. Care should be taken to ensure that the angle of insonation is less than 30°. It is important to check that the peak systolic velocity is greater than 60 cm/s to ensure that the uterine artery, rather than the arcuate artery, is being examined.

When three similar consecutive waveforms are obtained, the PI should be measured and the mean PI of the left and right arteries calculated by the software. Software automatically performs calculations, also MoMs, according to measurement data.

Pulsatility index = (Peak systolic velocity - minimum diastolic velocity) / Mean velocity

Step 5. Automatic risk assessment

The state of the art ASPRE algorithm developed by the Fetal Medicine Foundation

Specialized software generates a unique patient risk profile and report based on maternal risk factors, biochemistry test results and biophysical information.

Today's state of the art routine risk calculation software for pre-eclampsia uses the algorithm chosen for the ASPRE trial. This algorithm is using Bayes theorem to combine the prior risk with a number of selected biophysical and biochemical measurements. ^[15,16,34,43]

Combination of markers based on the availability

The marker combination used to screen for pre-eclampsia will depend on access and availability of the individual markers. If, for example, access to ultrasound is limited, it may not be possible to perform the UTPI measurement. ^[21] (see the right combination of markers on page 28)

The algorithm allows calculation of risks for pre-eclampsia based on maternal factors in combination with any of the compatible screening markers (PIGF, PAPP-A, MAP, UTPI). In first trimester screening, the time window for risk calculation is 11–13 weeks.

All markers, both biophysical and biochemical, have to be obtained within the same gestational age window.

Entering the screening data

Pre-eclampsia risk calculation software has an entry screen where information about a pregnant woman's medical history and risk factors, weight and height is transferred from the collection form (see an example at the end of this booklet, in Appendix).

Information about the blood sample collection date and when the specimen was received by the laboratory will be entered. If ultrasound and blood pressure were measured, the measurements and the date of those measurements will be added. Results from the blood test will be transferred to the same system as soon as they are available.

What should the cut off value be?

The risk cut off for preterm pre-eclampsia used in the ASPRE trial was 1:100.^[13] In other words, pregnancies in which the risk was calculated to be 1:100 and greater for preterm pre-eclampsia were categorized as high risk. This is also the default cut-off in risk calculation software such as PerkinElmer LifeCycle™ 6.0. software including the FMF ASPRE algorithm.

The latter software product allows the cut off to be configured to the desired detection rate or to the desired false positive rate, and different health systems and public health policy makers may choose other cut-off values in view of resource limitations and variations on the prevalence of pre-eclampsia in their populations.^[21]

The cut-off value used for preterm pre-eclampsia is likely to be very different to that commonly used in Down syndrome screening. The screen positive rate is often dependent on the next course of action. In Trisomy 21 screening the next course of action would be to offer an invasive diagnostic test, which carries a risk of miscarriage. For this reason, it is usually set at 5%.^[44] However, for pre-eclampsia

screening, the next course of action is to prescribe aspirin. As aspirin is considered safe, the key determinant is the % of women that can be managed to ensure compliance and follow-up. In the ASPRE trial this was deemed to be 10%.^[13]

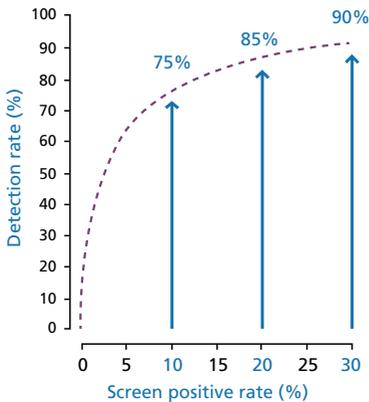
Table 3. Examples of cut-off values and the associated screen positive rates that might be used in combined pre-eclampsia screening and combined Trisomy 21 screening.

	Combined pre-eclampsia screening	Combined Trisomy 21 screening
Risk cut-off	1:100	1:250
Screen positive rate	10%	3–5%
Positives directed to	Aspirin treatment	Invasive testing

Table 3, intended for illustrative purposes only, includes cut off values that might be suitable for use in pre-eclampsia and Trisomy 21 screening respectively. Also shown are the screen positive rates that might then be expected.

The detection rate and screen positive rate go hand in hand and depend on the selected cut off. The detection rate and screen positive rate are always a compromise and the two examples below illustrate this.

Example 1– Aiming to identify a large number of high risk women e.g. with a detection rate of ~90%



By selecting a cut off for high DR, the screen positive rate will also increase (up to 30% in this example).^[45] This means that 30% of women will be prescribed aspirin if the ASPRE guideline is followed.

Figure 14. The ROC curve shows how detection rates go hand in hand with the screen positive rate and depend on the selected cut-off (blue arrows) for defining high risk for preterm pre-eclampsia (<37 weeks).^[45]

Example 2 – Aiming to keep the group of women taking aspirin during pregnancy small

By imposing a more stringent cut-off than in example 1, we will reduce the screen positives and consequently the number of women who will be prescribed aspirin. This will mean a reduced detection rate i.e. more of the affected pregnancies will be missed.

Patient report

PerkinElmer LifeCycle 6.0 software will generate a report where a woman's individual risk for developing pre-eclampsia is clearly presented and color coded (red=increased risk). The user can configure the report to show the risk for any or all of the pregnancy endpoints below:

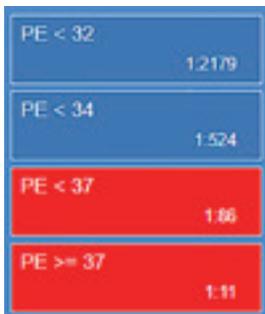


Figure 15. Example of the calculated risks on the LifeCycle 6.0 entry screen with 1:100 cut-off. Red indicates increased risk and blue low risk.

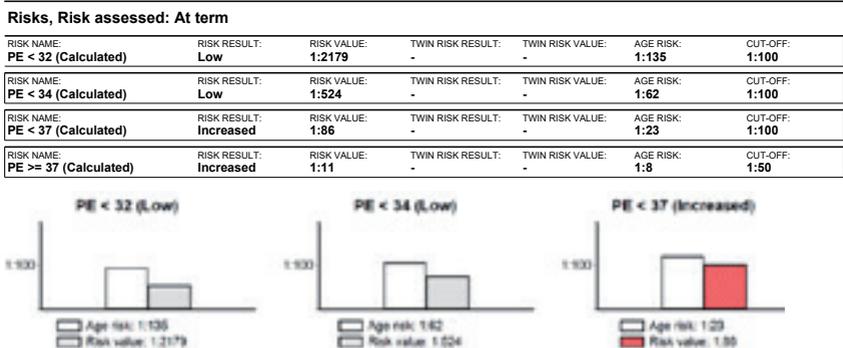


Figure 16. Example of a LifeCycle 6.0 patient report with result for very early pre-eclampsia (<32 weeks), preterm pre-eclampsia (<37 weeks) and term pre-eclampsia (≥ 37 weeks). Cut-offs and the risk end points (<32, <34, <37 and or ≥ 37 weeks) can be configured as desired.

11. The right combination of markers

Best results in pre-eclampsia screening are likely to be obtained by combining maternal history factors with the results from blood tests, blood pressure and ultrasound. Due to lack of local resources, it is not always possible to utilize all of the marker types. In settings with limited resources, combined pre-eclampsia screening can be implemented using combinations of those markers that are accessible. Different options, such as those suggested in Table 5, allow for local challenges in implementation.^[21, 46]

It is important to remember that screening using maternal characteristics and history together with a combination of available markers will invariably perform better than a simple screen using maternal factors alone.^[21]

In case there are local challenges in starting with the full protocol, variations of the combined test can be considered. However, maternal factors + MAP should be considered as the backbone of the prediction model.^[46]

Table 4. Screening options for different resource settings with performance estimates. FPR (False Positive Rate) = 10% in this example.^[37]

Method of screening	Detection rate		
	PE <34 w	PE <37 w	PE ≥37 w
Maternal factors	58%	50%	38%
Maternal factors plus:			
MAP	65%	60%	43%
MAP, UTPI	80%	70%	44%
MAP, PLGF	85%	73%	47%
MAP, UTPI, PLGF	90%	75%	47%

Section 3 – Management of screen positive pregnancies

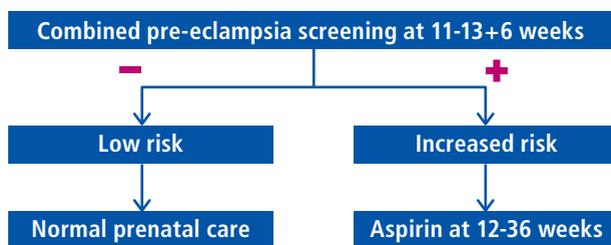


Figure 17. The pre-eclampsia screening algorithm.^[28]

12. Aspirin treatment according to ASPRE

Aspirin treatment is highly effective in the prevention of early (<34 weeks) and preterm pre-eclampsia (<37 weeks).^[12,13] Aspirin (150 mg daily, at bedtime) reduces the risk of preterm pre-eclampsia by more than 60% and the risk of early-onset pre-eclampsia (<34 weeks) by more than 80% when given to screen positive, high risk women, started at 11–14⁺⁶ weeks.

Table 5 summarizes the scientific background of treatment selected for the ASPRE trial.^[13]

Frequently asked questions on aspirin therapy

What is the treatment for screen positive women who are sensitive or allergic to aspirin?

As there is no other proven intervention, then expectant management would be appropriate. This would include frequent blood pressure measurements to ensure early diagnosis and anti-hypertensive drugs. Other potential prophylaxes, such as heparin and metformin, could be considered, depending on the reason for the categorization as high-risk.^[46]

Why not administer aspirin to all women?

Table 5. Aspirin treatment recommendations according to ASPRE. ^[13]

Dose	150 mg	The significant reduction in the rate of preterm pre-eclampsia is achieved using 150 mg of aspirin/night. ^[13, 47, 48] A high proportion (30%) of the population is non-responsive to aspirin if it is administered at doses lower than 100 mg (e.g. 75 mg or 81 mg). ^[29,49] A dose response effect of aspirin has been demonstrated, with higher daily doses leading to a greater risk reduction. ^[29,50]
When	Bed time	Aspirin taken at the end of the day is more effective in reducing the rate of pre-eclampsia than if taken in the morning or afternoon. ^[18]
Starting at	~12 weeks	Screen positive women benefit from aspirin treatment that is started early. ^[13,47] In practice, the starting time of the treatment is around 12 weeks, right after the results of the screening test are available. To be effective, the aspirin treatment must be started at the latest at 16 weeks. ^[12,13,29,47,51]
Finishing at	36 weeks	Women should continue to take aspirin up to 36 weeks of gestation. ^[13]

Firstly there would be a problem with compliance. It is highly unlikely that all pregnant women would agree to take 150 mg aspirin each night between 12 to 36 weeks without knowing their pre-eclampsia risk status.

The ASPRE trial has shown that the effectiveness of aspirin in prevention of preterm pre-eclampsia is compliance dependent (the more conscientiously the tablets are taken, the better the risk reduction). As an example, the compliance of pregnant women taking antenatal vitamins & minerals is only 49.2% and for folic acid it is only 19% ^[52,53]. If this level of compliance were achieved with daily aspirin, there would be virtually no benefit in combatting the problem of pre-eclampsia. The overall aspirin compliance among screen positive/high risk women in the ASPRE trial was 85%. ^[13]

A second issue is the possibility of side effects. Even though aspirin is considered as a safe drug, there are known side effects, such as upper gastrointestinal symptoms. ^[54] It is unnecessary to expose the entire pregnant population to these side effects without a clear indication of potential benefits. A woman suffering

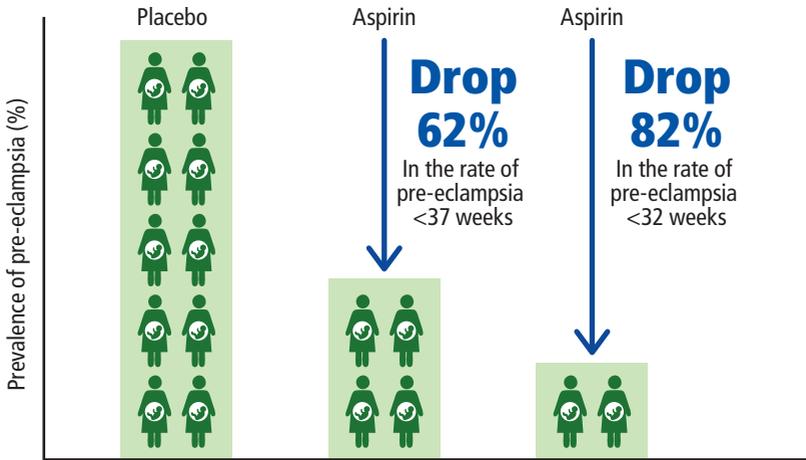


Figure 18 The ASPRE study has shown that aspirin is highly effective in reducing the prevalence of pre-eclampsia.

discomfort in her stomach due to aspirin, would likely to stop taking it, unless she knew she was at high risk of pre-eclampsia and that the benefits of the medicine were likely to outweigh the discomfort [46]. In addition, a study conducted in Italy in 2012 concluded that the use of aspirin was significantly associated with an increased risk of major gastrointestinal or cerebral bleeding episodes. [55]

Thirdly, the idea of unnecessary medication is worrying to many expecting parents. If universal aspirin prophylaxis were to be implemented, in a screened population of 10,000 women, 9,000 would be taking the drug unnecessarily. [46]

Does the same aspirin dose work with small and large women?

The ASPRE trial showed that daily aspirin administered at a dosage of 150 mg benefitted women over a wide range of BMI. The treatment effect size of aspirin was not different between BMI <25 vs BMI >25 kg/m². The rates of side effects were not significantly different between women with BMI <25 kg/m² and those with BMI ≥ 25 kg/m². There are no data to support the recommendation for a lower dosage. [46]

Section 4 – On informing parents about pre-eclampsia

13. What parents need to know

Whilst pre-eclampsia screening is critical to protect the health of the mother and the child, many women are unaware of pre-eclampsia as a disease or about the possibility to have their risk assessed with combined pre-eclampsia screening.^[56]

Women need to know that pre-eclampsia can affect any pregnancy. They should also be informed that some pregnancies are more at risk of pre-eclampsia than others, and that combined pre-eclampsia screening is an effective way to assess this risk.^[13]

To help make this kind of information available, PerkinElmer has prepared a pamphlet, *Pre-eclampsia screening – A guide for parents*.

Doctors have an important role in increasing the overall awareness of pre-eclampsia screening among women.

Pre-eclampsia screening and maternal anxiety

There is a concern that pre-eclampsia screening may cause increased maternal anxiety. This was examined in a recent study and no such concern was observed.^[57]

In fact, women with an increased risk of pre-eclampsia seem to be willing to engage in efforts to reduce their risk.

At a 10% screen positive rate for the new combined pre-eclampsia screening, there is no increase in anxiety over the widely accepted approach, using the NICE guidelines. Since the ASPRE algorithm will identify about 75% of women who will develop preterm pre-eclampsia (<37 weeks) whereas the NICE guideline would only identify 39%, it might be argued that there will be a net reduction in anxiety, due to the lower number of pregnancies that ultimately become pre-eclamptic.^[20,46]

Pre-screening information

Below are some of the questions asked by parents when confronted with the suggestion of pre-eclampsia screening, and some suggested answers.

How can pre-eclampsia affect me and the baby?

When pre-eclampsia develops, your blood pressure becomes elevated and protein in the urine may be present. The condition may affect the baby's growth and development and your own health. When pre-eclampsia occurs early in the pregnancy (before 37 weeks), the risk of early delivery and health problems in babies associated with premature delivery increases.

Am I at risk?

Pre-eclampsia is a relatively common complication of pregnancy affecting about 2–5% of pregnancies. All pregnancies have a small chance of complication related to the mother's risk factor profile.

Some pregnancies are at greater risk. Some of the factors that could indicate that your own risk may be higher are as follows

- You are pregnant for the first time, or even for the first time with your present partner
- You, your mother or your sister have had pre-eclampsia
- You have a body mass index (BMI) of 35 kg/m² or more
- You are over 40 years of age
- You are expecting twins, triplets or quadruplets
- You suffer from pre-existing high blood pressure, kidney problems, diabetes and/or autoimmune diseases – SLE/APS

Although the above risk factors are useful in assessing your pre-eclampsia risk, a far more reliable estimate can be made by your doctor using screening based on the PerkinElmer PIGF 1-2-3™ blood test in combination with uterine artery doppler measurement and mean arterial blood pressure measurement.

How can I reduce the risk of pre-eclampsia?

Taking aspirin daily before bedtime from 12 to 36 gestational weeks, as prescribed by your doctor, has been shown to be effective in reducing the rate of preterm pre-eclampsia. To get the best results, this treatment must be started sufficiently early, by week 16 at the very latest. You then need to take the medicine in the prescribed dose every evening through till week 36.

It is important to identify your risk of preterm pre-eclampsia as early as possible in the pregnancy with a screening test. Then there will be adequate time for your doctor to ensure that aspirin treatment is started at the right time.

When should I be screened for pre-eclampsia?

You should be screened for pre-eclampsia at 11–13⁺⁶ weeks' gestation.

How is pre-eclampsia screening performed?

A detailed antenatal history is taken to identify known risk factors. Your weight and height are measured and mean arterial blood pressure measurement is performed. A blood sample is taken for PIGF or PAPP-A analysis and an ultrasound examination is performed to determine the UTPI.^[13]

What does the test tell you?

The test tells you whether you are high risk or low risk. High risk women will benefit from aspirin treatment.

What is the benefit of testing for preterm pre-eclampsia ?

Finding out the condition if you are at high risk of pre-eclampsia is the first step to delaying or preventing it. Delaying pre-eclampsia helps prevent premature births and gives your baby a healthy start to life.

Post-screening information

Below are some of the questions possibly asked by parents when confronted with the information that a pregnancy is classified as high risk for pre-eclampsia. While the answers, quite properly, are reassuring, it should be clearly conveyed that pre-eclampsia is a serious disorder that can affect the lifetime health of both mother and child. The parents must understand the importance of avoidance.

What do the pre-eclampsia screening results mean?

If you are at low risk, you are unlikely to develop pre-eclampsia later in your pregnancy. You will continue to receive normal prenatal monitoring and counseling.

If your risk is increased, you will not necessarily develop pre-eclampsia, but your doctor would suggest that you start taking the recommended dose of aspirin as a preventative measure. The use of aspirin to prevent pre-eclampsia should always be discussed with a health care professional.

What is the treatment?

Treatment with aspirin (150 mg/day) taken at bed time from weeks 12 to 36 of the pregnancy has shown to reduce the risk of preterm pre-eclampsia by more than 60%.^[13,58] The aspirin dose should be at minimum 100 mg/day.^[29] As an example, if only 81mg tablet is commercially available, then 162 mg would be the recommended dose.

If your doctor prescribes aspirin, it is important to take your medication regularly every night. Results have shown that taking 90% of the tablets achieves 70% reduction in the rate of preterm pre-eclampsia.^[17,28]

What is known about the safety of aspirin?

The safety data on aspirin are reassuring. There is no evidence that aspirin increases vaginal spotting. It is suggested that aspirin at a daily dose of ≥ 100 mg initiated at ≤ 16 weeks may also decrease the risk of adverse events other than pre-eclampsia (placental abruption or antepartum hemorrhage).^[29]

Appendix



Clinical information form PIGF 1-2-3™ PRE-ECLAMPSIA SCREENING TEST

Laboratory stamp/Logo:

PATIENT DETAILS		LABORATORY DETAILS	
First name: <input type="text"/>	Last name: <input type="text"/>	Laboratory: <input type="text"/>	Division: <input type="text"/>
Dr: <input type="text"/>	Telephone: <input type="text"/>	Laboratory address: <input type="text"/>	
Date of birth: <input type="text"/>		Postal code: <input type="text"/>	City: <input type="text"/>
Address: <input type="text"/>		Telephone: <input type="text"/>	Requester ID: <input type="text"/>
Postal code: <input type="text"/>	City: <input type="text"/>		

PATIENT BACKGROUND			
Ethnicity: <input type="checkbox"/> White/Caucasian <input type="checkbox"/> Asian <input type="checkbox"/> African <input type="checkbox"/> Hispanic <input type="checkbox"/> Other	Gest date: <input type="text"/>	Number of fetuses*: <input type="text"/>	Multi-day diabetes: <input type="checkbox"/> No <input type="checkbox"/> Yes
Height (cm): <input type="text"/>	DOB: <input type="text"/>	Smoker: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Diabetes: <input type="checkbox"/> No <input type="checkbox"/> Yes
Weight (kg): <input type="text"/>	Medication date: <input type="text"/>	Marriage(s): <input type="checkbox"/> No <input type="checkbox"/> Yes	Multi-treatment for type II diabetes: <input type="checkbox"/> No <input type="checkbox"/> Yes
		<small>*This covers pre-eclampsia or eclampsia or other chronic conditions in the past.</small>	

PREGNANCY			
Conception method: <input type="checkbox"/> Spontaneous <input type="checkbox"/> Ovulation drugs <input type="checkbox"/> In vitro fertilisation	Anti-phospholipid syndrome: <input type="checkbox"/> No <input type="checkbox"/> Yes	First preg. pre-eclampsia: <input type="checkbox"/> No <input type="checkbox"/> Yes	First preg. delivery date: <input type="text"/>
	Systemic lupus erythematosus: <input type="checkbox"/> No <input type="checkbox"/> Yes	Last preg. preg. gest. at delivery (w-o/d): <input type="text"/>	Chronic hypertension: <input type="checkbox"/> No <input type="checkbox"/> Yes
	Maternal or paternal had pre-eclampsia: <input type="checkbox"/> No <input type="checkbox"/> Yes	Inter-pregnancy interval (years): <input type="text"/>	
	First no. of pregnancies*: <input type="text"/>	First preg. baby weight (kg): <input type="text"/>	
	*Spontaneous or IVF		

BIOCHEMISTRY			
Sample ID: <input type="text"/>	Date specimen collected: <input type="text"/>	Specimen received: <input type="text"/>	Weight (kg): <input type="text"/>
Gen. at sample date (w-o/d): <input type="text"/>	Specimen notes: <input type="text"/>	Baby/mom notes: <input type="text"/>	Test: <input type="checkbox"/> BUN-A <input type="checkbox"/> BUN

BLOOD PRESSURE									
Gen. at sample date (w-o/d): <input type="text"/>	Specimen notes: <input type="text"/>	Time measured: <input type="text"/>	Weight at the measuring date (kg): <input type="text"/>						
TEST	TEST DATA	LEFT DIAST. #1	LEFT DIAST. #2	LEFT DIAST. #3	LEFT DIAST. #4	RIGHT DIAST. #1	RIGHT DIAST. #2	RIGHT DIAST. #3	RIGHT DIAST. #4
BUN	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

ULTRASOUND			
Transducer (MHz): <input type="text"/>	CR: <input type="text"/>	TEST	LEFT UTERINE AREA
Scan date: <input type="text"/>	Specimen notes: <input type="text"/>	RFI	RIGHT UTERINE AREA
Gen. at sample date (w-o/d): <input type="text"/>	Weight at scan date (kg): <input type="text"/>	<input type="text"/>	<input type="text"/>
Gen. at manual entry (w-o/d): <input type="text"/>			

Figure 20. An example of pre-eclampsia patient information collection form.

References

1. Tranquilli AL, et al., The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertens* 2014; 4: 97-104.
2. Verghese L, et al., Antenatal screening for pre-eclampsia: evaluation of the NICE and pre-eclampsia community guidelines. *J Obstet Gynaecol.* 2012;32(2):128–131
3. World Health Organization. Make Every Mother and Child Count. World Health Report, 2005. Geneva, Switzerland: World Health Organization; 2005.
4. Kuklina EV, et al. Hypertensive disorders and severe obstetric morbidity in the United States. *Obstet Gynecol* 2009; 113:1299-306
5. Wu P et al., Preeclampsia and Future Cardiovascular Health: A Systematic Review and Meta-Analysis. *Circ Cardiovasc Qual Outcomes* 2017; 10.
6. Veerbeek JH et al., Cardiovascular disease risk factors after early-onset preeclampsia, late-onset preeclampsia, and pregnancy-induced hypertension. *Hypertension* 2015; 65: 600-606.
7. Parker S et al. Updated National Birth Prevalence estimates for selected birth defects in the United States, 2004-2006. *Birth Defects Res A Clin Mol Teratol.* 2010,
8. Duley L. The global impact of pre-eclampsia and eclampsia. *Semin Perinatol* 2009,
9. Romo A et al. Intrauterine growth retardation (IUGR): epidemiology and etiology. *Pediatr Endocrinol Rev.* 2009,
10. WHO media centre, fact sheet 2016. <http://www.who.int/mediacentre/factsheets/fs363/en/>
11. Stevens et. al. Short-term costs of preeclampsia to the United States health care system *AJOG* 2017
12. Roberge S et al., Early administration of low-dose aspirin for the prevention of preterm and term preeclampsia: a systematic review and meta-analysis. *Fetal Diagn Ther.* 2012;31(3):141-6
13. Rolnik DL et al., ASPRE trial; Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *N Engl J Med.* 2017 Aug 17;377(7):613-622
14. Poon LC et al. First-Trimester Prediction of Hypertensive Disorders in Pregnancy Hypertension 2009; 53: 812-818 Melchiorre, Sharma, Thilaganathan. Cardiovascular implications in preeclampsia. *Circulation.* 2014 Aug 19;130(8):703-14.

15. Wright D et al., A competing risks model in early screening for preeclampsia. *Fetal Diagn Ther.* 2012;32(3):171-8.
16. Wright D et al., Competing risks model in screening for preeclampsia by maternal characteristics and medical history. *Am J Obstet Gynecol* 2015; 213: 62.e1-10.
17. Wright D et al. Aspirin for Evidence-Based Preeclampsia Prevention trial: influence of compliance on beneficial effect of aspirin in prevention of preterm preeclampsia. *Am J Obstet Gynecol.* 2017 Sep 6
18. Ayala DE et al., Chronotherapy with low-dose aspirin for prevention of complications in pregnancy. *Chronobiol Int* 2013; 30: 260-279.
19. Wright D. Et al. Aspirin for Evidence-Based Pre-eclampsia Prevention trial: effect of aspirin on length of stay in the neonatal intensive care unit. "American journal of obstetrics and gynecology." *Am J Obstet Gynecol.* 2018 Mar 2.
20. O’Gorman et al. Multicenter screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks gestation: comparison to NICE guidelines and ACOG recommendations *Ultrasound Obstet Gynecol* 2017
21. Rolnik DL et al., Early screening and prevention of preterm pre-eclampsia with aspirin: time for clinical implementation. *Ultrasound Obstet Gynecol.* 2017 Nov;50(5):551-556
22. ACOG. First-trimester risk assessment for early-onset preeclampsia. Committee opinion No. 638. *Obstet Gynecol* 2015; 126: e25-7. American College of Obstetricians and Gynecologists. Task Force on hypertension in pregnancy. The American College of Obstetricians and Gynecologists; Washington (DC): 2013; 122: 1122–1131.
23. National Collaborating Centre for Women's and Children's Health (UK). Hypertension in Pregnancy: The Management of Hypertensive Disorders During Pregnancy. London: RCOG Press, 2010.
24. Tsiakkas A et al., Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; 45: 591-8.
25. Wright A, et al., Maternal serum PAPP-A and free β -hCG at 12, 22 and 32 weeks' gestation in screening for pre-eclampsia. *Ultrasound Obstet Gynecol* 2016; 47: 762-7.
26. Poon LC, et al., Protocol for measurement of mean arterial pressure at 11-13 weeks' gestation. *Fetal Diagn Ther* 2012; 31: 42-8.
27. O’Gorman N et al., Uterine artery pulsatility index at 12, 22, 32 and 36 weeks' gestation in screening for pre-eclampsia. *Ultrasound Obstet Gynecol* 2016; 47: 565-72.

28. FMF Courses: pre-eclampsia screening - <https://courses.fetalmedicine.com/fmf/show/826?locale=en>
29. Roberge S et al., Aspirin for the prevention of preterm and term preeclampsia: Systematic review and meta-analysis. *Am J Obstet Gynecol.* 2017 Nov 11.
30. Kypros Nicolaides, webcast Q&A 2017
31. National Collaborating Centre for Women's and Children's Health. Antenatal Care: Routine Care for the Healthy Pregnant Woman. Clinical Guideline. Commissioned by the National Institute for Clinical Excellence. London, United Kingdom: RCOG Press; 2008.
32. PerkinElmer PIGF assay product inserts 13908271, 13907817
33. Cuckle, H.S. Screening for pre-eclampsia – lessons from aneuploidy screening. *Placenta* 2011; , 32, Suppl:S42-48.
34. Akolekar, R et al., Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. *Fetal Diagn. Ther.*, 33, 8-15.
35. Poon, L.C.Y et al., First trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. *Ultrasound Obstet. Gynecol* 2009 ., 33, 23-33.
36. Wright A et al., Maternal serum PAPP-A and free β -hCG at 12, 22 and 32 weeks' gestation in screening for pre-eclampsia. *Ultrasound Obstet Gynecol* 2016; 47: 762-7.
37. O'Gorman et al., Accuracy of competing risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation. *Ultrasound Obstet Gynecol* Jun 2017.
38. Tan MY et. al., Screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol* 2018; 52; 186-195.
39. PerkinElmer sample handling guide (internal)
40. Reinders et al , An accurate automated blood pressure device for use in pregnancy and pre-eclampsia: the Microlife 3BTO-A. *BJOG* 2005
41. http://www.dableducational.org/sphygmomanometers/devices_2_sbpm.html#ArmTable
42. Hannah L Nathan et al ., Blood pressure measurement in pregnancy. *The Obstetrician & Gynaecologist.* 2015;17:91–8
43. O'Gorman et al., Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks gestation. *Am J Obstet Gynecol.* 2016 Jan;214(1):103
44. EUROCAT special report 2010 Prenatal Screening Policies in Europe

45. Pre-eclampsia presentation –Liona Poon ISSHP Berlin 2017
46. ASPRE Q&A – answers provided by K. Nicolaides & L..Poon.
47. Rolnik DL, et al, ASPRE trial: performance of screening for preterm pre-eclampsia. *Ultrasound Obstet Gynecol.* 2017 Oct;50(4)
48. Beaufils et al., Prevention of pre-eclampsia by early antiplatelet therapy. *Lancet* 1985
49. Caron N et al, Low-dose ASA response using the PFA-100 in women with high-risk pregnancy. *J Obstet Gynaecol Can.* 2009 Nov;31(11):1022-7.
50. Roberge S et al., The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis. *Am J Obstet Gynecol.* 2017 Feb;216(2):110-120.e6.
51. Bujold E et al., Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. *Obstet Gynecol* 2010; 116: 402-414.
52. Thirukkanesh and A.M. Zahara., Compliance to Vitamin and Mineral Supplementation among Pregnant Women in Urban and Rural Areas in Malaysia. *PJN* 2010,
53. McNulty B et al., Women's compliance with current folic acid recommendations and achievement of optimal vitamin status for preventing neural tube defects. *Hum Reprod.* 2011 Jun;26(6):1530-6
54. <https://www.rxlist.com/aspirin-side-effects-drug-center.htm>
55. De Berardis G et al. Association of aspirin use with major bleeding in patients with and without diabetes. *JAMA* 2012 Jun 6;307(21):2286-94
56. Pre-eclampsia awareness survey 2014, pre-eclampsia foundation
57. Simeone S, Lojo C et al., Psychological impact of first-trimester prevention for preeclampsia on anxiety. *Prenat Diagn* 2015; 35: 60-64.
58. Supplement to: Rolnik DL, Wright D, Poon LC, et al. Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia. *N Engl J Med* 2017;377:613-22.

Products may not be licensed in accordance with the laws in all countries, such as the United States and Canada. Please check with your local representative for availability

PerkinElmer, Inc.
9440 Winter Street
Waltham, MA 0244544 USA
Phone: (800) 762-44000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer, Inc.
Wallac Oy
PO Box 10
20101 Turku, Finland
Phone: + 358 2 2678111
Fax: + 358 2 2678 357

ISO 13485 · ISO 9001 · CDMCAS · ISO 14001 · OHSAS 18001



For a complete listing of our global offices, visit www.perkinelmer.com/lasoffices

© 2018 PerkinElmer, Inc. All rights reserved. PerkinElmer is a registered trademark of PerkinElmer, Inc. All trademarks depicted are the property of their respective holders or owners. PerkinElmer reserves the right to change this document at any time and disclaims liability for editorial, pictorial or typographical errors.