**Clinical Performance Study Validation Plan**

**B·R·A·H·M·S Fast Screen PLUS 1.0 Software**

Product number: 107853

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# Scope

This document specifies the planned data analysis for the evaluation of the clinical performance of B·R·A·H·M·S Fast Screen PLUS.

# Background

B·R·A·H·M·S Fast Screen PLUS is a risk assessment software for the non-invasive screening of fetal trisomy 21 (T21), fetal trisomy 18 (T18) and fetal trisomy 13 (T13) in the first trimester of pregnancy, as well as T21, T18 and fetal neural tube defects (NTD) in the second trimester of pregnancy. B·R·A·H·M·S Fast Screen PLUS is also a software to be used in non-invasive first, second and third trimester screening for the risk of developing pre-eclampsia (PE).

B·R·A·H·M·S Fast Screen PLUS software combines in one interface prediction models to create a user-friendly application for risk calculation in clinical practice. All prediction models implemented in B·R·A·H·M·S Fast Screen PLUS and the algorithms on which they are based were established either by the Fetal Medicine Foundation Germany (FMF-DE) or by the Fetal Medicine Foundation United Kingdom (FMF-UK) and were validated in large prospective clinical studies.

The FMF-UK is a Registered Charity that aims to improve the health of pregnant women and their babies through research and training in fetal medicine. The FMF-UK has developed risk prediction models for various pregnancy complications based on large clinical studies and provides access to their prediction models (e.g., via online calculators, https://www.fetalmedicine.org/research/assess/preeclampsia/first-trimester). The FMF-DE is a non-profit organization that offers to all interested pregnant women in the first trimester of pregnancy prenatal diagnostics that go beyond the usual maternity care and meet the highest quality standards. The FMF-DE has established a first trimester prediction model based on an extensive database of clinical data (186.215 pregnancy data sets collected by FMF-DE between 2003 and 2013) and provides access to the prediction model via the commercially available prenatal risk calculation (PRC3.0) software.

# Description Software

The B·R·A·H·M·S Fast Screen PLUS software consists of seven prediction models that can be used to estimate the risk of a pregnancy being affected by T21, T18, T13, NTD or the development of PE at different trimesters (publications containing a description of the prediction models are summarized in Table 1).

Table 1: Algorithms of B·R·A·H·M·S Fast Screen PLUS with corresponding literature references to implemented prediction models

| Algorithm ID | Trimester | Risk assessment for | Source of prediction model | Reference of  prediction model |
| --- | --- | --- | --- | --- |
| 1\_FMF\_DE | 1st (1T) | T21, T18/T13 | FMF-DE | Merz et al., 20161 |
| 2\_FMF\_UK | 1st (1T) | T21, T18, T13 | FMF-UK | Wald et al., 20032  Kagan et al., 20083  Wright et al., 20104  Santorum et al., 20175 |
| 3\_FMF\_UK | 2nd (2T) | T21, T18, NTD | FMF-UK | Wald et al., 20032  Spencer et al., 19996  Wald et al., 19777 |
| 4\_FMF\_UK | 1st & 2nd (1T&2T) | T21, T18 | FMF-UK | Wald et al., 20032  Spencer et al., 19996 |
| 5\_FMF\_UK | 1st (1T) | Development of PE | FMF-UK | O'Gorman et al., 20178  Tan et al., 20189 |
| 6\_FMF\_UK | 2nd (2T) | Development of PE | FMF-UK | Litwinska et al., 201810 |
| 7\_FMF\_UK | 3rd (3T) | Development of PE | FMF-UK | Panaitescu et al., 201811 Tsiakkas et al., 201612  Andrietti et al., 201613 |

The prediction models for the screening of fetal T21, T18, T13 and NTD abnormalities are algorithms which use Bayes’ theorem to update prior probabilities obtained from maternal age and previous pregnancy characteristics, with biomarker measurements. The models to predict the development of PE are algorithms which use a competing risk model based on Bayes’ theorem to estimate individual patient-specific risks. This involves calculating an estimate of the a priori risk based on the maternal factors and an estimate of the adjusted risk based on the combination of the maternal factors with the results of the measurements of other parameters. An overview of the parameters for the biophysical, ultrasound and biochemical measurements and the possible combination of the parameters for each prediction model are summarized in Figure 1.

Ein Bild, das Text, Screenshot, Zahl, Schrift enthält.

Automatisch generierte Beschreibung

Figure 1: Structure of the B·R·A·H·M·S Fast Screen PLUS software architecture indicating the possible parameter combinations of the different screening algorithms. Parameters for first trimester screening are shown in blue, parameters for second trimester screening are shown in orange, parameters for third trimester screening are shown in red.

# Experimental Methods and Statistical Principles

## Rationale for validation

To create a user-friendly application for risk calculation in clinical practice, B·R·A·H·M·S Fast Screen PLUS software combines in a single interface four algorithms for T21, T18, T13 and NTD screening and three algorithms to screen for the risk of PE development. All algorithms were established either by the FMF-DE or by the FMF-UK and were validated extensively in large prospective clinical studies in the past decades14,15. The purpose of the clinical validation of the B·R·A·H·M·S Fast Screen PLUS software is to use clinical data to evaluate whether the algorithms have been successfully implemented in the software.

## Data collection

In the past, numerous clinical studies with B·R·A·H·M·S prenatal screening biomarker assays were conducted 9,16.Based on these studies, B·R·A·H·M·S’ clinical cooperation partners provided data sets which will be used for the clinical validation of B·R·A·H·M·S Fast Screen PLUS. Apart from the measurement results of the biomarkers the clinical data sets contain anonymized patient information on maternal characteristics, sonographic and biophysical measurements as required by the respective algorithms.

For a few rare parameter combinations that allow screening for fetal trisomy, real clinical data sets including B·R·A·H·M·S prenatal biomarker assays were not available. In these cases, artificial datasets consisting of simulated data were provided by the FMF-DE and the FMF-UK. The FMF-DE provided a simulated dataset that can be used to compare the risks calculated by 1\_FMF\_DE of B·R·A·H·M·S Fast Screen PLUS with risks calculated by its reference system. The FMF-UK provided a simulated dataset that can be used to determine the performance measures (Chapter 4.5.) of the algorithms 2\_FMF\_UK, 3\_FMF\_UK and 4\_FMF\_UK, as this dataset resembles real data. Simulated datasets mimicking real data have been used in the past to determine the performance of trisomy screening algorithms.17

## Cleaning of clinical data

Prior to risk assessment with B·R·A·H·M·S Fast Screen PLUS, the clinical records are checked for completeness of input parameters. For example, if the required information on the mother's date of birth is missing, the date will be calculated using the mother's age and the date of clinical examination. For other missing input variables, the default settings of B·R·A·H·M·S Fast Screen PLUS will be used (see IFU, HN-CUS-4225).

## Clinical data sets

An overview of the clinical and simulated data sets used for clinical validation of the B·R·A·H·M·S Fast Screen PLUS software is shown in Table 2.

Table 2: Data sets for clinical validation of B·R·A·H·M·S Fast Screen PLUS

|  |  |  |  |
| --- | --- | --- | --- |
| Cohort ID | Data type | Source | Algorithm ID |
| 1T\_Triso\_FMF\_UK | real clinical | FMF UK, D. Wright | 1\_FMF\_DE  2\_FMF\_UK |
| 1T\_Triso\_RF\_Quad | real clinical | FMF, King’s College UK | 1\_FMF\_DE  2\_FMF\_UK |
| 1T\_Triso\_Canada | real clinical | CHU de Québec, E. Bujold | 1\_FMF\_DE  2\_FMF\_UK |
| 2T\_Triso\_ T21 | real clinical | Sheffield Teaching Hospital, UK | 3\_FMF\_UK |
| 2T\_Triso\_T18 | real clinical | King George Hospital, UK, Stuart Jones | 3\_FMF\_UK |
| 2T\_Triso\_NTD | real clinical | University Hospital Timone, Marseille, France | 3\_FMF\_UK |
| 12T\_Triso\_ T21 | real clinical | CHU Brest, France | 4\_FMF\_UK |
| T1\_PE\_FMF UK | real clinical | FMF UK, A. Wright | 5\_FMF\_UK |
| T1\_PE\_Canada | real clinical | CHU de Québec, E. Bujold | 5\_FMF\_UK |
| T1\_PE\_twins | real clinical | FMF UK, A. Wright | 5\_FMF\_UK |
| T2\_PE\_FMF UK | real clinical | FMF UK, A. Wright | 6\_FMF\_UK |
| T3\_PE\_30\_34\_Canada | real clinical | CHU de Québec, E. Bujold | 7\_FMF\_UK |
| T3\_PE\_35\_37\_FMF\_UK | real clinical | FMF UK, A. Wright | 7\_FMF\_UK |
| Sim\_add\_FMF\_DE | simulated | FMF DE, C. Thode | 1\_FMF\_DE |
| Sim\_twins\_FMF\_DE | simulated | FMF DE, C. Thode | 1\_FMF\_DE |
| Triso\_single\_sim | simulated | FMF UK, D. Wright | 4\_FMF\_UK |
| Triso\_twin\_sim | simulated | FMF UK, D. Wright | 2\_FMF\_UK  3\_FMF\_UK  4\_FMF\_UK |

## Clinical performance testing

For the validation of the clinical performance of B·R·A·H·M·S Fast Screen PLUS, the seven algorithms will be tested using existing clinical data sets that were collected as part of clinical studies. The software will be used to calculate a risks per patient, and the performance of the software to predict clinical outcomes will be determined per validation data set. For each algorithm clinical performance measures will be determined for binary risk estimates, i.e., risks above a specific cut-off will be considered as “positive” and risks below the cut-off as “negative”. The risk cut-off is either specified directly, e.g., as 1:100, or indirectly by a pre-specified false positive rate, e.g., 10%. Using a contingency table of test results (binary risks, levels: negative or positive) vs. outcome results (levels: pregnancy affected or not affected), the number of true positives, false positives, false negatives, and true negatives will be reported. The success of the validation will be determined in accordance with pre-specified acceptance criteria for each algorithm, which are defined in the validation planning (Chapter 4.6)

Clinical performance measures will be determined according to CLSI EP12-Ed3. Confidence intervals will be calculated according to the Wilson score method17. The following performance measures will be used:

1. Detection Rate (DR, sensitivity): proportion of affected pregnancies with a positive test result.

DR = # true positives / (# true positives + # false negatives)

1. False positive rate (FPR): proportion of unaffected pregnancies with a positive test result. The FPR is calculated for clinical data sets.

FPR = # false positives / (# true negatives + # false positives)

1. Screen positive rate (SPR): proportion of positive results of the total number of screenings performed. The SPR is calculated for simulated data sets.

SPR = (# true positives + # false positives) / (# true positives + # false positives + # true negatives + # false negatives)

1. Specificity: proportion of unaffected pregnancies with a negative test result.

Specificity = # true negatives / (# true negatives + # false positives) = 1 - FPR

1. Positive predicted value (PPV): probability of affected pregnancy given a positive test result.

PPV = # true positives / (# true positives + # false positives)

= DR × prevalence / (DR × prevalence + FPR × (1 - prevalence))

1. Negative predicted value (NPV): probability of unaffected pregnancy given a negative test result.

NPV = # true negatives / (# true negatives + # false negatives)

= specificity × (1 - prevalence)/ (specificity × (1 - prevalence) + (1 - DR) × prevalence)

1. Odds of being affected given a positive test result (OAPR): ratio of the number of affected to unaffected pregnancies among pregnancies with positive test results, i.e., ratio of the number of true-positives to the number of false-positives among pregnancies with positive test results.

OAPR = (DR / FPR) x prevalence

## Validation planning

### Algorithm 1\_FMF\_DE: Trisomy screening 1st trimester

#### Description

The algorithm 1\_FMF\_DE was developed by the FMF-DE and is used for 1st trimester screening to assess the risk for trisomies T21 and for both, T18+T13 (combined risk). The algorithm requires maternal factors (MFs), nuchal translucency (NT), and biomarker data for pregnancy associated plasma protein A (PAPP-A) and Free β human Chorionic Gonadotropin (Free ßhCG) for risk assessment. The pregnancy dating, which can be assessed by crown-rump length (CRL), date of in vitro fertilization (IVF) or other methods, is also a mandatory parameter. As additional input parameters, nasal bone (NB), tricuspid regurgitation (TR) and ductus venosus flow (DV) may be used in this algorithm. The mandatory and optional input parameters for the algorithm 1\_FMF\_DE are listed in Table 3. The algorithm requires that the ultrasound examination is performed at gestational age of 11+1 to 14+0 weeks and the blood sample for biochemical markers is obtained at gestational age 9+0 to 14+0 weeks.

The measured concentrations of free ßhCG and PAPP-A need to be standardized as they depend on gestational age and maternal characteristics such as weight, ethnicity, and others. For standardization the FMF DE algorithm converts the measured values into “Degree of Extremeness” (DoE)1. DoE considers the deviation from the median and the upper (95th percentile) or lower (5th percentile) of the norm, respectively. The output of the software is a risk estimate reported as a 1:X value. The various reported risks depend on the combination of input parameters and are listed in Table 4.

Table 3: Input parameters for algorithm 1\_FMF\_DE

| Input | Type | Parameters |
| --- | --- | --- |
| Mandatory | MFs | Patient consent |
| Mandatory | MFs | Identifier (e.g. patient ID) |
| Mandatory | MFs | Maternal date of birth |
| Mandatory | MFs | Weight |
| Mandatory | MFs | Geographical origin |
| Mandatory | MFs | Smoking status |
| Mandatory | MFs | Diabetes status |
| Mandatory | MFs | Pregnancy dating |
| Mandatory | MFs | Method of conception |
| Mandatory | Ultrasound | Date of ultrasound examination |
| Mandatory | Ultrasound | Twin pregnancy |
| Mandatory | Ultrasound | Date of ultrasound examination |
| Mandatory if pregnancy dating is CRL | Ultrasound | CRL |
| Mandatory | Ultrasound | NT |
| Optional | Ultrasound | NB |
| Optional | Ultrasound | DV |
| Optional | Ultrasound | TR |
| Mandatory | Biochemical | Date of blood sampling |
| Mandatory | Biochemical | Sample ID |
| Mandatory | Biochemical | Free ßhCG |
| Mandatory | Biochemical | PAPP-A |

Table 4: Outputs of algorithm 1\_FMF\_DE

|  |  |  |  |
| --- | --- | --- | --- |
| Output | Description | T21 | T18/13 |
| Background risk | Prior risk, obtained from MFs | x | x |
| Biochemical risk | MFs + PAPP-A + Free ßhCG | x | x |
| NT risk\* | MFs + NT | x | x |
| Adjusted risk | final risk calculated by software based on  all input parameters | x | x |

\*only reported for IVF conception or twin pregnancy or “vanishing” twin pregnancy

#### Analysis settings

Table 5 describes the combinations of parameters to be used for performance assessment. The performance measures of Chapter 4.5 will be computed for each combination of parameters.

For the simulated data sets the risks will be calculated by the 1\_FMF\_DE algorithm and compared with its reference algorithm by Passing-Bablok regression.

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Table 5: Parameter combinations for performance assessment of the 1\_FMF\_DE algorithm

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Analysis ID | Data Type | Parameters | T21 | T18/13 |
| 1\_FMF\_DE\_A1 | Clinical | MFs, NT, PAPP-A, Free ß hCG | x | x |
| 1\_FMF\_DE\_A2 | Clinical | MFs, NT, PAPP-A, Free ß hCG, NB | x | x |
| 1\_FMF\_DE\_A3 | Simulated | MFs, NT, PAPP-A, Free ß hCG, NB, TR, DV | x | x |

#### Validation cohorts

Cohorts used for validation were either derived from clinical cohorts (Table 6) or simulated data (Table 7). The three clinical cohorts listed in Table 6 will be combined into one clinical cohort for validation testing.

Table 6: Clinical cohorts for validation of the 1\_FMF\_DE algorithm and number of patients

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # T21 | # T18 | # T13 |
| 1T\_Triso\_FMF\_UK | MFs, NT, PAPP-A, Free ß hCG | 512 | 442 | 41 | 15 | 14 |
| 1T\_Triso\_RF\_Quad | MFs, NT, PAPP-A, Free ß hCG | 374 | 298 | 50 | 18 | 8 |
| 1T\_Triso\_Canada | MFs, NT, PAPP-A, Free ß hCG, NB | 1374 | 1337 | 24 | 7 | 6 |

Table 7: Simulated data for validation of the 1\_FMF\_DE algorithm and number of patients

|  |  |  |  |
| --- | --- | --- | --- |
| Cohort ID | Parameters | # Singleton pregnancies | # Twin pregnancies |
| Sim\_add\_FMF\_DE | MFs, NT, PAPP-A, Free ß hCG, NB, TR, DVPI | 30 | 0 |
| Sim\_twins\_FMF\_DE | MFs, NT, PAPP-A, Free ß hCG, NB, TR, DVPI | 0 | 15 |

#### Acceptance criteria for validation

URP: FSP\_05\_01\_01\_01

A DR > 70% and a FPR < 7% will be considered as validation success for the 1\_FMF\_DE algorithm with the parameters NT, PAPP-A and Free ß hCG for prediction of pregnancies affected by (i) T21 and (ii) T18/13. A fixed risk cut-off 1:150 will be used.

DR testing hypotheses are:

Null hypothesis H0: DR ≤ 70%

Alternative hypothesis H1:  DR > 70%

FPR testing hypotheses are:

Null hypothesis H0: FPR ≥ 7%

Alternative hypothesis H1:  FPR < 7%

One-sided statistical testing will be conducted by exact test for one proportion on α = 2.5% statistical significance level. Statistical power > 90% was estimated for all the testing scenarios based on available sample sizes and assumed true performances taken from literature (Table 8):

* DR of T21: 115 cases, 85% assumed true DR
* FPR of T21: 2077 unaffected pregnancies, 3% assumed true FPR
* DR of T18/T13: 68 cases, 85% assumed true DR
* FPR of T18/T13: 2077 unaffected pregnancies, 2% assumed true FPR

Table 8: References of diagnostic performance assumptions used for power calculations for the 1\_FMF\_DE algorithm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | Parameter | Indication | Cut-off | DR | FPR |
| Merz et al., 20161 | MFs, NT, PAPP-A, Free ßhCG | T21 | 1:150 | 86.8% | 3.42% |
| Merz et al., 20161 | MFs, NT, PAPP-A, Free ßhCG | T18/T13 | 1:150 | 86.4% | 1.60% |

#### Performance reporting

URP: FSP\_05\_01\_01\_01

Point estimates and 95% confidence intervals will be reported for the performance measures described in Chapter 4.5. DR point estimates are expected to be similar to the corresponding DR estimates reported in literature (Table 8).

### Algorithm 2\_FMF\_UK: Trisomy screening 1st trimester

#### Description

The algorithm 2\_FMF\_UK was developed by the FMF-UK and is used for 1st trimester screening to assess the risk for trisomies T21, T18 and T13. The algorithm requires maternal factors (MFs), NT, PAPP-A and Free ßhCG for risk assessment. The gestational age (GA) on the day of the examination, which can be assessed by crown-rump length (CRL), date of in vitro fertilization (IVF) or other methods (GA direct), is also a mandatory parameter. As additional parameters Placental Growth Factor (PlGF), Alpha Fetoprotein (AFP), NB, Ductus Venosus Pulsatility Index (DVPI) and Fetal Heart Rate (FHR) may be used. The required and optional input parameters for the algorithm 2\_FMF\_UK are listed in Table 9. The algorithm requires that the ultrasound examination is performed at gestational age of 11+2 to 14+1 weeks and the blood sample for biochemical markers is obtained at gestational age 8+0 to 14+0 weeks.

The measured concentrations of the biomarkers PAPP-A, Free ßhCG, PlGF and AFP need to be standardized as they depend on gestational age and maternal characteristics such as weight, ethnicity, and others. For standardization the FMF-UK algorithm converts the measured values into so-called “Multiples of Median” (MoM) values. The outputs of the software are estimated risks reported as a 1:X value (Table 10).

Table 9: Input parameters for the 2\_FMF\_UK algorithm

| Input | Type | Parameters |
| --- | --- | --- |
| Mandatory |  | Patient consent |
| Mandatory |  | Identifier (e.g. patient ID) |
| Mandatory | MFs | Maternal date of birth |
| Mandatory | MFs | Weight |
| Mandatory | MFs | Geographical origin |
| Mandatory | MFs | Smoking status |
| Mandatory | MFs | Diabetes status |
| Mandatory | MFs | Assessment of GA (Pregnancy dating) |
| Mandatory | MFs | Method of conception |
| Mandatory | MFs | Parity |
| Mandatory if parous | MFs | Interpregnancy interval |
| Mandatory if parous | MFs | Pre-eclampsia in previous pregnancy |
| Mandatory if parous | MFs | Previous chromosomal anomalies |
| Mandatory | Ultrasound | Date of ultrasound examination |
| Mandatory | Ultrasound | Twin pregnancy |
| Mandatory | Ultrasound | Crown rump length (CRL) |
| Mandatory | Ultrasound | NT |
| Optional | Ultrasound | NB |
| Optional | Ultrasound | DVPI |
| Optional | Ultrasound | FHR |
| Mandatory | Biochemical | Date of blood sampling |
| Mandatory | Biochemical | Sample ID |
| Mandatory | Biochemical | Free ßhCG |
| Mandatory | Biochemical | PAPP-A |
| Optional | Biochemical | PlGF |
| Optional | Biochemical | AFP |

Table 10: Outputs of the 2\_FMF\_UK algorithm

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Output | Description | T21 | T18 | T13 |
| Background risk | Prior risk, obtained from MFs | x | x | x |
| Adjusted risk | Final risk calculated by software based on all the input parameters (also named “posterior risk”) | x | x | x |

#### Analysis settings

Table 11 lists the combinations of parameters to be used for performance assessment of the 2\_FMF\_UK algorithm. The performance measures of Chapter 4.5 will be computed for each combination of parameters. The possible combinations of biomarkers in the 2\_FMF\_UK algorithm require a total of 11 analyses. To be able to process the required analyses more efficiently during validation, the analyses were ranked on the following criteria:

(1) frequency in clinical practice (often used parameter combination ranked higher than less used parameter combination),

(2) type of data (real clinical data sets ranked higher than simulated data sets),

(3) type of parameters (mandatory parameters ranked higher than optional parameters),

(4) number of parameters (fewer parameters ranked higher than more parameters).

Table 11: Parameter combinations for performance assessment of the 2\_FMF\_UK algorithm

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Analysis ID | Analysis Rank | Data Type | Parameters | T21 | T18 | T13 |
| 2\_FMF\_UK\_A1 | 1 | Clinical | MFs, NT, PAPP-A, Free ß hCG | x | x | x |
| 2\_FMF\_UK\_A2 | 8 | Clinical | MFs, NT, PAPP-A, Free ß hCG, PlGF | x | x | x |
| 2\_FMF\_UK\_A3 | 5 | Clinical | MFs, NT, PAPP-A, Free ß hCG, NB | x | x | x |
| 2\_FMF\_UK\_A4 | 6 | Clinical | MFs, NT, PAPP-A, Free ß hCG, DVPI | x | x | x |
| 2\_FMF\_UK\_A5 | 7 | Clinical | MFs, NT, PAPP-A, Free ß hCG, FHR | x | x | x |
| 2\_FMF\_UK\_A6 | 3 | Simulated\* | MFs, NT, PAPP-A, Free ß hCG, PlGF, AFP | x | x | x |
| 2\_FMF\_UK\_A7 | 9 | Simulated\* | MFs, PAPP-A, Free ß hCG, PlGF, AFP | x | x | x |
| 2\_FMF\_UK\_A8 | 2 | Simulated\*\* | MFs, NT, PAPP-A, Free ß hCG | x | x | x |
| 2\_FMF\_UK\_A9 | 10 | Simulated\*\* | MFs, NT, PAPP-A, Free ß hCG, PlGF | x | x | x |
| 2\_FMF\_UK\_A10 | 4 | Simulated\*\* | MFs, NT, PAPP-A, Free ß hCG, PlGF, AFP | x | x | x |
| 2\_FMF\_UK\_A11 | 11 | Simulated\*\* | MFs, PAPP-A, Free ß hCG, PlGF, AFP | x | x | x |

\*only singelton pregnancies (Triso\_single\_sim)

\*\*only twin pregnancies (Triso\_twin\_sim)

#### Validation cohorts

Cohorts used for validation were either derived from clinical cohorts (Table 12) or simulated (Table 13). The three clinical cohorts listed in Table 12 will be combined into one clinical cohort for validation testing.

Table 12: Clinical cohorts for validation of 2\_FMF\_UK algorithm

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # T21 | # T18 | # T13 |
| 1T\_Triso\_FMF\_UK | MFs, NT, PAPP-A, Free ß hCG | 512 | 442 | 41 | 15 | 14 |
| 1T\_Triso\_RF\_Quad | MFs, NT, PAPP-A, Free ß hCG | 374 | 298 | 50 | 18 | 8 |
| 1T\_Triso\_Canada | MFs, NT, PAPP-A, Free ß hCG, PlGF, NB, FHR, DVPI | 1374 | 1337 | 24 | 7 | 6 |

Table 13: Simulated data for validation of 2\_FMF\_UK algorithm

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # T21 | # T18 | # T13 |
| Triso\_single\_sim | MFs, NT, PAPP-A, Free ß hCG, PlGF, AFP, NB, FHR, DVPI | 10.000 | 2.500 | 2.500 | 2.500 | 2.500 |
| Triso\_twin\_sim | MFs, NT, PAPP-A, Free ß hCG, PlGF, AFP, NB, FHR, DVPI | 10.000 | 2.500 | 2.500 | 2.500 | 2.500 |

#### Acceptance criteria for validation

URP: FSP\_05\_01\_02\_01

The following endpoint-specific diagnostic performances will be considered as validation success for the 2\_FMF\_DE algorithm with the parameters NT, PAPP-A and Free ß hCG:

1. T21: a DR > 70% and a FPR < 8% at a fixed cut-off 1:100
2. T18: a DR > 70% and a FPR < 6% at a fixed cut-off 1:50
3. T13: a DR > 55% and a FPR < 6% at a fixed cut-off 1:50

For T21 and T18 DR testing hypotheses are:

Null hypothesis H0: DR ≤ 70%

Alternative hypothesis H1:  DR > 70%

For T13 DR testing hypotheses is:

Null hypothesis H0: DR ≤ 55%

Alternative hypothesis H1:  DR > 55%

For T21 FPR testing hypotheses is:

Null hypothesis H0: FPR ≥ 8%

Alternative hypothesis H1:  FPR < 8%

For T18 and T13 FPR testing hypotheses are:

Null hypothesis H0: FPR ≥ 6%

Alternative hypothesis H1:  FPR < 6%

One-sided statistical testing will be conducted by exact test for one proportion on α = 2.5% statistical significance level. Statistical power > 90% was estimated for all the testing scenarios based on available sample sizes and assumed true performances taken from literature (Table 14):

* DR of T21: 115 cases, 91% assumed true DR
* FPR of T21: 2077 unaffected pregnancies, 5% assumed true FPR
* DR of T18: 40 cases, 95% assumed true DR
* FPR of T18: 2077 unaffected pregnancies, 2% assumed true FPR
* DR of T13: 28 cases, 88% assumed true DR
* FPR of T13: 2077 unaffected pregnancies, 2% assumed true FPR

Table 14: References for power calculation assumptions to the 2\_FMF\_UK algorithm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | Parameters | Indication | Cut-off | DR  (95% CI) | FPR  (95% CI) |
| Santorum et al., 20175 | MFs, NT, PAPP-A, Free ßhCG | T21 | 1:100 | 91.2%  (88.1-93.7) | 4.7%  (4.6-4.8) |
| Santorum et al., 20175 | MFs, NT, PAPP-A, Free ßhCG | T18 | 1:50 | 94.6%  (90.0-97.5) | 2.7% (2.6-2.8) |
| Santorum et al., 20175 | MFs, NT, PAPP-A, Free ßhCG | T13 | 1:50 | 87.5%  (75.9-94.8) | 2.7% (2.6-2.8) |

#### Performance reporting

URP: FSP\_05\_01\_02\_01

From the analysis of the clinical and simulated datasets, point estimates and 95% confidence intervals will be reported for performance measures described in Chapter 4.5. The determined DR point estimates of the validation are expected to be similar to the DR estimates reported in literature (Table 15).

Table 15: Reference performance measures for 2\_FMF\_UK algorithm

| Reference | Parameters | Indication | Cut-off | DR  (95% CI) | FPR  (95% CI) |
| --- | --- | --- | --- | --- | --- |
| Santorum et al., 20175 | MFs, NT, PAPP-A, Free ßhCG | T21 | 1:100 | 91.2%  (88.1-93.7) | 4.7%  (4.6-4.8) |
| Santorum et al., 20175 | MFs, NT, PAPP-A, Free ßhCG | T18 | 1:50 | 94.6%  (90.0-97.5) | 2.7% (2.6-2.8) |
| Santorum et al., 20175 | MFs, NT, PAPP-A, Free ßhCG | T13 | 1:50 | 87.5%  (75.9-94.8) | 2.7% (2.6-2.8) |
| Santorum et al., 20175 | MFs, NT, PAPP-A, Free ßhCG, FHR | T21 | 1:100 | 92.1%  (89.2-94.5) | 5.3%  (5.2-5.4) |
| Santorum et al., 20175 | MFs, NT, PAPP-A, Free ßhCG, FHR | T18 | 1:50 | 94.6%  (90.0-97.5) | 3.4% (3.3-3.5) |
| Santorum et al., 20175 | MFs, NT, PAPP-A, Free ßhCG, FHR | T13 | 1:50 | 91.1%  (80.4-97.0) | 3.4% (3.3-3.5) |
| Kagan et al., 2009 19 | MFs, NT, PAPP-A, Free ßhCG, NB | T21 | 1:100 | 92% | 2.9% |
| Kagan et al., 2009 19 | MFs, NT, PAPP-A, Free ßhCG, NB | T18 | 1:50 | 93% | 1.4% |
| Kagan et al., 2009 19 | MFs, NT, PAPP-A, Free ßhCG, NB | T18 | 1:50 | 83% | 1.4% |
| Maiz et al., 201220 | MFs, NT, PAPP-A, Free ßhCG, DVPI | T21 | 1:100 | 93.5%  (90.8-96.2) | 1.6% (1.5 -1.7) |
| Caron et al., 202321 | MFs, NT, PAPP-A, Free ß hCG, PlGF, AFP | T21 | 1:300 | 96% | 4.4% |
| Caron et al., 202321 | MFs, PAPP-A, Free ß hCG, PlGF, AFP | T21 | 1:300 | 88% | 13.2% |

### Algorithm 3\_FMF\_UK: Trisomy screening 2nd trimester

#### Description

The algorithm 3\_FMF\_UK was developed by the FMF-UK and is applied for 2nd trimester screening to assess the risk for trisomies T21 and T18 using MFs in combination with two to four biochemical markers. The double test includes AFP and Free ßhCG or hCGß, the triple test AFP, Free ßhCG or hCGß and uE3, and the quadruple test AFP, Free ßhCG or hCGß, uE3 and InhibinA. In addition, this algorithm is used to screen for neural tube defects (NTD) based on the calculated MoM value of AFP. The mandatory and optional input parameters for the algorithm 3\_FMF\_UK are listed in Table 16. The algorithm requires that the blood sample for biomarkers is obtained at gestational age 14+0 to 19+6 weeks for the double, the triple, or the quadruple test and at gestational age 15+0 to 19+6 weeks for screening of NTD.

The measured concentrations of the biomarkers AFP, Free ßhCG or hCGß, uE3 and InhibinA need to be standardized as they depend on gestational age and maternal characteristics, for example such as weight, ethnicity, and others. For standardization the FMF-UK algorithm converts the measured values into so-called Multiples of Median (MoMs) values. The outputs of the software are estimated risks reported as a 1:X value. The output of the software for NTD screening is the calculated AFP MoM value (Table 17).

Table 16: Input parameters for algorithm 3\_FMF\_UK

| Input | Type | Parameters |
| --- | --- | --- |
| Mandatory |  | Patient consent |
| Mandatory |  | Identifier (e.g. patient ID) |
| Mandatory | MFs | Maternal date of birth |
| Mandatory | MFs | Weight |
| Mandatory | MFs | Geographical origin |
| Mandatory | MFs | Smoking status |
| Mandatory | MFs | Diabetes status |
| Mandatory | MFs | Pregnancy dating |
| Mandatory | Ultrasound | Date of ultrasound examination |
| Mandatory | Ultrasound | Twin pregnancy |
| Mandatory | Biochemical | Date of blood sampling |
| Mandatory | Biochemical | Sample ID |
| Mandatory | Biochemical | AFP |
| Mandatory | Biochemical | Free ßhCG or hCGß |
| Optional | Biochemical | uE3 |
| Optional | Biochemical | InhibinA |

Table 17: Outputs of the algorithm 3\_FMF\_UK

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Output | Description | T21 | T18 | NTD |
| Background risk | Prior risk, obtained from MFs | x | x | x |
| Adjusted risk | Risk calculated by software based on all input parameters | x | x\* |  |
| MoM AFP | MoM AFP values ≥ 2.5 are highlighted |  |  | x |

\* In case of quad test, risk assessment on triple test is reported by the software

#### Analysis settings

Table 18 lists the combinations of parameters to be used for performance assessment of the 3\_FMF\_UK algorithm. The performance measures of Chapter 4.5 will be computed for each combination of parameters. The possible combinations of biomarkers in the 3\_FMF\_UK algorithm require a total of 12 analyses. To be able to process the required analyses more efficiently during validation, the analyses were ranked on the following criteria:

(1) frequency in clinical practice (often used parameter combination ranked higher than less used parameter combination),

(2) type of data (real clinical data sets ranked higher than simulated data sets),

(3) type of parameters (mandatory parameters ranked higher than optional parameters),

(4) number of parameters (fewer parameters ranked higher than more parameters).

Table 18: Combinations of parameters for performance assessment of the 3\_FMF\_UK algorithm

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Analysis ID | Analysis Rank | Data Type | Test Type | Parameter Combinations | T21 | T18 | NTD |
| 3\_FMF\_UK\_A1 | 2 | Clinical | Double | MFs, AFP, Free ß hCG | x | x | x |
| 3\_FMF\_UK\_A2 | 3 | Clinical | Double | MFs, AFP, hCGß | x | x | x |
| 3\_FMF\_UK\_A3 | 6 | Clinical | Triple | MFs, AFP, Free ß hCG, uE3 | x | x | x |
| 3\_FMF\_UK\_A4 | 7 | Clinical | Triple | MFs, AFP, hCGß, uE3 | x | x | x |
| 3\_FMF\_UK\_A5 | 1 | Clinical | Quad | MFs, AFP, Free ß hCG, uE3, InhibinA | x | \* | x |
| 3\_FMF\_UK\_A6 | 5 | Clinical | Quad | MFs, AFP, hCGß, uE3, InhibinA | x | \* | x |
| 3\_FMF\_UK\_A7 | 8 | Simulated | Double | MFs, AFP, Free ß hCG | x | x | x |
| 3\_FMF\_UK\_A8 | 9 | Simulated | Double | MFs, AFP, hCGß | x | x | x |
| 3\_FMF\_UK\_A9 | 11 | Simulated | Triple | MFs, AFP, Free ß hCG, uE3 | x | x | x |
| 3\_FMF\_UK\_A10 | 12 | Simulated | Triple | MFs, AFP, hCGß, uE3 | x | x | x |
| 3\_FMF\_UK\_A11 | 4 | Simulated | Quad | MFs, AFP, Free ß hCG, uE3, InhibinA | x | \* | x |
| 3\_FMF\_UK\_A12 | 10 | Simulated | Quad | MFs, AFP, hCGß, uE3, InhibinA | x | \* | x |

\*risks of triple test is reported

#### Validation cohorts

Table 19 lists the clinical study cohorts that are available for performance assessment. The three clinical study cohorts will be combined into one clinical cohort for validation of the B·R·A·H·M·S Fast Screen PLUS software. Table 20 lists the simulated data sets that are available for performance assessment.

Table 19: Clinical data for validation of the 3\_FMF\_UK algorithm

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # T21 | # T18 | # NTD |
| 2T\_Triso\_ T21 | MFs, AFP, Free ß hCG, hCGß, uE3, InhibinA | 509 | 446 | 69 | 0 | 0 |
| 2T\_Triso\_T18 | MFs, AFP, Free ß hCG, hCGß, uE3, InhibinA | 15 | 0 | 0 | 15 | 0 |
| 2T\_Triso\_NTD | MFs, AFP, Free ß hCG | 7 | 0 | 0 | 0 | 7 |

Table 20: Simulated data for validation of the 3\_FMF\_UK algorithm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # T21 | # T18 |
| Triso\_twin\_sim | MFs, AFP, Free ß hCG, hCGß, uE3, InhibinA | 7.500 | 2.500 | 2.500 | 2.500 |

#### Acceptance criteria for validation

URP-FSP\_05\_01\_03\_02/ 03, URP-FSP\_05\_01\_04\_02

A DR > 60% will be considered as validation success for the 3\_FMF\_UK algorithm with the parameters AFP, Free ß hCG, uE3, Inhibin A for prediction of pregnancies affected by T21. The DR will be computed at a pre-specified FPR of 5% determined on the respective validation cohort.

Testing hypotheses are:

Null hypothesis H0: DR ≤ 60%

Alternative hypothesis H1:  DR > 60%

One-sided statistical testing will be conducted by exact test for one proportion on α = 2.5% statistical significance level. Statistical power > 90% was estimated for T21 assuming 69 available T21 affected pregnancies and a true DR of 81%. Published DRs were used as the basis for the assumed true DR (Table 21).

Due to the limited availability of clinical data for pregnancies affected by T18 or NTD one-sided statistical testing is regarded not adequate for these endpoints (only 15 T18 affected pregnancies and only 7 NTD affected pregnancies are available, this would result in insufficiently informative testing). Accordingly, validation success will already be considered (1) if the DR for the 15 T18 cases according to adjusted risks is larger than or equal to the DR according to the corresponding prior risks at a fixed FPR of 2% and (2) if 4 of the 7 NTD affected pregnancies have an AFP MoM value above or equal to the cut-off 2.5.

Table 21: References of diagnostic performance assumptions used for power calculation for the 3\_FMF\_UK algorithm

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Reference | Test | Parameters | Indication | Cut-off | DR | FPR |
| Wald et al., (2003)2 | Quad | MFs, AFP, Free ßhCG, uE3, InhibinA | T21 |  | 83% | 5% |
| Palomaki et al., (1995)22 | Triple | MFs, AFP, hCGß, uE3 | T18 |  | 81% | 2% |
| Wald et al., (1977)7 | Double, Triple, Quad | AFP MoM | NTD | ≥ 2.5 | 69% |  |

#### Performance reporting

URP-FSP\_05\_01\_03\_01/ 02/ 03, URP-FSP\_05\_01\_04\_02

Point estimates and 95% confidence intervals will be reported for the performance measures described in Chapter 4.5 for clinical study data and for simulated data sets. DR point estimates are expected to be similar to the corresponding DR estimates reported in literature (Table 22).

Table 22: Performance estimates of the 3\_FMF\_UK algorithm reported in literature

| Reference | Test | Parameters | Indication | Cut-off | DR | FPR |
| --- | --- | --- | --- | --- | --- | --- |
| Wald et al., (2003)2 | Double | MFs, AFP, Free ßhCG | T21 |  | 71% | \*5% |
| Wald et al., (2003)2 | Double | MFs, AFP, Free ßhCG | T21 | \*1:205 | 75% |  |
| Wald et al., (2003)2 | Double | MFs, AFP, hCGß | T21 |  | 66% | \*5% |
| Spencer et al., (1993)23 | Double | MFs, AFP, Free ßhCG | T18 |  | 50% | \*1% |
| Palomaki et al., (1995)22 | Double | MFs, AFP, hCGß | T18 |  | 55% | \*2% |
| Wald et al., (2003)2 | Triple | MFs, AFP, Free ßhCG, uE3 | T21 |  | 77% | \*5% |
| Wald et al., (2003)2 | Triple | MFs, AFP, Free ßhCG, uE3 | T21 | \*1:160 | 75% |  |
| Wald et al., (2003)2 | Triple | MFs, AFP, hCGß, uE3 | T21 |  | 74% | \*5% |
| Palomaki et al., (1995)22 | Triple | MFs, AFP, hCGß, uE3 | T18 |  | 81% | \*2% |
| Palomaki et al., (1995)22 | Triple | MFs, AFP, hCGß, uE3 | T18 | \*1:100 | 60% | 0.2% |
| Wald et al., (2003)2 | Quad | MFs, AFP, Free ßhCG, uE3, InhibinA | T21 |  | 83% | \*5% |
| Wald et al., (2003)2 | Quad | MFs, AFP, Free ßhCG, uE3, InhibinA | T21 | \*1:175 | 80% |  |
| Wald et al., (2003)2 | Quad | MFs, AFP, hCGß, uE3, InhibinA | T21 |  | 81% | \*5% |
| Wald et al., (1977)7 | Double Triple Quad | AFP MoM | NTD | \*≥ 2.5 | 69% | 5% |

\*fixed value to determine DR

### Algorithm 4\_FMF\_UK: Trisomy screening 1st and 2nd trimester

#### Description

The algorithm 4\_FMF\_UK was developed by the FMF-UK and is applied for 1st and 2nd integrated or sequential screening to assess the risk for T21 and T18 using MFs in combination with parameters measured in the 1st and 2nd trimester. Table 23 listed all possible marker combinations of this algorithm.

Table 23: Possible marker combinations of the 4\_FMF\_UK algorithm

| Indication | 1st trimester parameters | 2nd trimester parameters | 2nd trimester test |
| --- | --- | --- | --- |
| T21, T18 | NT | MFs, AFP, Free ßhCG | Double |
| T21, T18 | NT | MFs, AFP, hCGß | Double |
| T21, T18 | NT | MFs, AFP, Free ßhCG, uE3 | Triple |
| T21, T18 | NT | MFs, AFP, hCGß, uE3 | Triple |
| T21 | NT | MFs, AFP, Free ßhCG, uE3, inhibinA | Quad |
| T21 | NT | MFs, AFP, hCGß, uE3, inhibinA | Quad |
| T21 | PAPP-A | MFs, AFP, Free ßhCG | Double |
| T21 | PAPP-A | MFs, AFP, hCGß | Double |
| T21 | PAPP-A | MFs, AFP, Free ßhCG, uE3 | Triple |
| T21 | PAPP-A | MFs, AFP, hCGß, uE3 | Triple |
| T21 | PAPP-A | MFs, AFP, Free ßhCG, uE3, inhibinA | Quad |
| T21 | PAPP-A | MFs, AFP, hCGß, uE3, inhibinA | Quad |
| T21 | NT, PAPP-A | MFs, AFP, Free ßhCG | Double |
| T21 | NT, PAPP-A | MFs, AFP, hCGß | Double |
| T21 | NT, PAPP-A | MFs, AFP, Free ßhCG, uE3 | Triple |
| T21 | NT, PAPP-A | MFs, AFP, hCGß, uE3 | Triple |
| T21 | NT, PAPP-A | MFs, AFP, Free ßhCG, uE3, inhibinA | Quad |
| T21 | NT, PAPP-A | MFs, AFP, hCGß, uE3, inhibinA | Quad |

The required input parameters for the algorithm 4\_FMF\_UK are listed in Table 24. The algorithm requires that the ultrasound examination is performed at the gestational age of 11+2 to 14+1 weeks and the blood sample for biochemical markers is obtained at the gestational age of 8+0 to 14+1 weeks in the first trimester and at the gestational age of 14+0 to 19+6 weeks in the second trimester.

The measured concentrations of the biomarkers PAPP-A, AFP, Free ßhCG or hCGß, uE3 and InhibinA need to be standardized as they depend on gestational age and maternal characteristics such as weight, ethnicity and others. For standardization the FMF-UK algorithm converts the measured values into so-called Multiples of Median (MoMs) values. The outputs of the software are estimated risks reported as a 1:X value (Table 25).

Table 24: Input parameters for the 4\_FMF\_UK algorithm

|  |  |  |
| --- | --- | --- |
| Input | Type | Parameters |
| Mandatory |  | Patient consent |
| Mandatory |  | Identifier (e.g. patient ID) |
| Mandatory | MFs | Maternal date of birth |
| Mandatory | MFs | Weight |
| Mandatory | MFs | Geographical origin |
| Mandatory | MFs | Smoking status |
| Mandatory | MFs | Diabetes status |
| Mandatory | MFs | Assessment of GA (Pregnancy dating) |
| Mandatory | MFs | Method of conception |
| Mandatory | Ultrasound | Date of ultrasound examination |
| Mandatory | Ultrasound | Twin pregnancy |
| Mandatory | Ultrasound | Crown rump length (CRL) |
| Optional | Ultrasound | Nuchal Translucency (NT) |
| Optional | Biochemical T1 | Sample ID |
| Optional | Biochemical T1 | Date of blood sampling |
| Optional | Biochemical T1 | PAPP-A |
| Mandatory | Biochemical T2 | Sample ID |
| Mandatory | Biochemical T2 | Date of blood sampling |
| Mandatory | Biochemical T2 | AFP |
| Mandatory | Biochemical T2 | Free ßhCG or hCGß |
| Optional | Biochemical T2 | uE3 |
| Optional | Biochemical T2 | InhibinA |

Table 25: Outputs of the 4\_FMF\_UK algorithm

|  |  |  |  |
| --- | --- | --- | --- |
| Output | Description | T21 | T18 |
| Background risk | Prior risk, obtained from MFs | x | x |
| Adjusted risk | Risk calculated by software based on input parameters | x | x |

#### Analysis settings

Only one combination of parameters could be covered by the clinical dataset for the 4\_FMF\_UK algorithm, as T18 and T21 cases occur very rarely in the 1st and 2nd integrated or sequential screening. Therefore, mainly simulated data were used to evaluate the performance of the algorithm. The Table 26 lists the combinations of parameters to be used for performance assessment of the 4\_FMF\_UK algorithm. The performance measures of Chapter 4.5 will be computed for each combination of parameters. The possible combinations of biomarkers in the 4\_FMF\_UK algorithm require a total of 37 analyses. To be able to process the required analyses more efficiently during validation, the analyses were ranked on the following criteria:

(1) frequency in clinical practice (often used parameter combination ranked higher than less used parameter combination),

(2) type of data (real clinical data sets ranked higher than simulated data sets),

(3) type of parameters (mandatory parameters ranked higher than optional parameters),

(4) number of parameters (fewer parameters ranked higher than more parameters).

Table 26: Parameters for the determining of performance measures of the 4\_FMF\_UK algorithm

| Analysis ID | Analysis Rank | Data set type | 1T Parameter Combinations | 2T Parameter Combinations | T21 | T18 |
| --- | --- | --- | --- | --- | --- | --- |
| 4\_FMF\_UK\_A1 | 1 | Clinical | NT | MFs, AFP, Free ß hCG | x | x |
| 4\_FMF\_UK\_A2 | 2 | Simulated\* | NT | MFs, AFP, Free ß hCG | x | x |
| 4\_FMF\_UK\_A3 | 3 | Simulated\* | NT | MFs, AFP, hCGß | x | x |
| 4\_FMF\_UK\_A4 | 26 | Simulated\* | NT | MFs, AFP, Free ßhCG, uE3 | x | x |
| 4\_FMF\_UK\_A5 | 27 | Simulated\* | NT | MFs, AFP, hCGß, uE3 | x | x |
| 4\_FMF\_UK\_A6 | 6 | Simulated\* | NT | MFs, AFP, Free ßhCG, uE3, inhibinA | x | \* |
| 4\_FMF\_UK\_A7 | 7 | Simulated\* | NT | MFs, AFP, hCGß, uE3, inhibinA | x | \* |
| 4\_FMF\_UK\_A8 | 18 | Simulated\* | PAPP-A | MFs, AFP, Free ß hCG | x | - |
| 4\_FMF\_UK\_A9 | 19 | Simulated\* | PAPP-A | MFs, AFP, hCGß | x | - |
| 4\_FMF\_UK\_A10 | 30 | Simulated\* | PAPP-A | MFs, AFP, Free ßhCG, uE3 | x | - |
| 4\_FMF\_UK\_A11 | 31 | Simulated\* | PAPP-A | MFs, AFP, hCGß, uE3 | x | - |
| 4\_FMF\_UK\_A12 | 10 | Simulated\* | PAPP-A | MFs, AFP, Free ßhCG, uE3, inhibinA | x | - |
| 4\_FMF\_UK\_A13 | 11 | Simulated\* | PAPP-A | MFs, AFP, hCGß, uE3, inhibinA | x | - |
| 4\_FMF\_UK\_A14 | 22 | Simulated\* | NT, PAPP-A | MFs, AFP, Free ß hCG | x | - |
| 4\_FMF\_UK\_A15 | 23 | Simulated\* | NT, PAPP-A | MFs, AFP, hCGß | x | - |
| 4\_FMF\_UK\_A16 | 34 | Simulated\* | NT, PAPP-A | MFs, AFP, Free ßhCG, uE3 | x | - |
| 4\_FMF\_UK\_A17 | 34 | Simulated\* | NT, PAPP-A | MFs, AFP, hCGß, uE3 | x | - |
| 4\_FMF\_UK\_A18 | 14 | Simulated\* | NT, PAPP-A | MFs, AFP, Free ßhCG, uE3, inhibinA | x | - |
| 4\_FMF\_UK\_A19 | 15 | Simulated\* | NT, PAPP-A | MFs, AFP, hCGß, uE3, inhibinA | x | - |
| 4\_FMF\_UK\_A20 | 4 | Simulated\*\* | NT | MFs, AFP, Free ß hCG | x | x |
| 4\_FMF\_UK\_A21 | 5 | Simulated\*\* | NT | MFs, AFP, hCGß | x | x |
| 4\_FMF\_UK\_A22 | 28 | Simulated\*\* | NT | MFs, AFP, Free ßhCG, uE3 | x | x |
| 4\_FMF\_UK\_A23 | 29 | Simulated\*\* | NT | MFs, AFP, hCGß, uE3 | x | x |
| 4\_FMF\_UK\_A24 | 8 | Simulated\*\* | NT | MFs, AFP, Free ßhCG, uE3, inhibinA | x | \* |
| 4\_FMF\_UK\_A25 | 9 | Simulated\*\* | NT | MFs, AFP, hCGß, uE3, inhibinA | x | \* |
| 4\_FMF\_UK\_A26 | 20 | Simulated\*\* | PAPP-A | MFs, AFP, Free ß hCG | x | - |
| 4\_FMF\_UK\_A27 | 21 | Simulated\*\* | PAPP-A | MFs, AFP, hCGß | x | - |
| 4\_FMF\_UK\_A28 | 32 | Simulated\*\* | PAPP-A | MFs, AFP, Free ßhCG, uE3 | x | - |
| 4\_FMF\_UK\_A29 | 33 | Simulated\*\* | PAPP-A | MFs, AFP, hCGß, uE3 | x | - |
| 4\_FMF\_UK\_A30 | 12 | Simulated\*\* | PAPP-A | MFs, AFP, Free ßhCG, uE3, inhibinA | x | - |
| 4\_FMF\_UK\_A31 | 13 | Simulated\*\* | PAPP-A | MFs, AFP, hCGß, uE3, inhibinA | x | - |
| 4\_FMF\_UK\_A32 | 24 | Simulated\*\* | NT, PAPP-A | MFs, AFP, Free ß hCG | x | - |
| 4\_FMF\_UK\_A33 | 25 | Simulated\*\* | NT, PAPP-A | MFs, AFP, hCGß | x | - |
| 4\_FMF\_UK\_A34 | 36 | Simulated\*\* | NT, PAPP-A | MFs, AFP, Free ßhCG, uE3 | x | - |
| 4\_FMF\_UK\_A35 | 37 | Simulated\*\* | NT, PAPP-A | MFs, AFP, hCGß, uE3 | x | - |
| 4\_FMF\_UK\_A36 | 16 | Simulated\*\* | NT, PAPP-A | MFs, AFP, Free ßhCG, uE3, inhibinA | x | - |
| 3\_FMF\_UK\_A37 | 17 | Simulated\*\* | NT, PAPP-A | MFs, AFP, hCGß, uE3, inhibinA | x | - |

\*risks of triple test is reported

#### Validation cohorts

Cohorts used for validation were either derived from clinical cohorts (Table 27) or simulated (Table 28).

Table 27: Clinical data for validation of the 4\_FMF\_UK algorithm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # T21 | # T18 |
| 12T\_Triso\_ T21 | 1T: NT  2T: MFs, AFP, Free ß hCG | 110 | 106 | 4 | 0 |

Table 28: Simulated data for validation of the 4\_FMF\_UK algorithm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # T21 | # T18 |
| Triso\_single\_sim | 1T: NT, PAPP-A  2T: MFs, AFP, Free ß hCG, hCGß, uE3, InhibinA | 7.500 | 2.500 | 2.500 | 2.500 |
| Triso\_twin\_sim | 1T: NT, PAPP-A  2T: MFs, AFP, Free ß hCG, hCGß, uE3, InhibinA | 7.500 | 2.500 | 2.500 | 2.500 |

#### Acceptance criteria for validation

URP-FSP\_05\_01\_05\_03

Due to the limited availability of clinical data for pregnancies affected by T21 or T18 one-sided statistical testing is regarded not adequate for these endpoints (only 4 T21 affected pregnancies and none T18 affected pregnancies are available, this would result in insufficiently informative testing). Accordingly, validation success will already be considered if the DR for the 4 T21 cases according to adjusted risks is larger than or equal to the DR according to the corresponding prior risks at a fixed FPR of 5%.

#### Performance reporting

URP-FSP\_05\_01\_05\_03/ 04/ 05/ 06/ 07/ 08/ 09/ 11

Point estimates and 95% confidence intervals will be reported for the performance measures described in Chapter 4.5 for clinical study data and for simulated data sets. DR point estimates are expected to be similar to the corresponding DR estimates reported in literature (Table 29).

Table 29: Performance estimates of the 4\_FMF\_UK algorithm reported in literature

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | 1T Parameters | 2T Parameters | Indication | DR | FPR |
| Wald et al., 20032 | MFs, NT | MFs, AFP, Free ßhCG | T21 | 87% | 5% |
| Wald et al., 20032 | MFs, NT | MFs, AFP, hCGß | T21 | 86% | 5% |
| Spencer et al., (1993)23 |  | MFs, AFP, Free ßhCG | T18 | 50% | 1% |
| Palomaki et al., (1995)22 |  | MFs, AFP, hCGß | T18 | 55% | 2% |
| Wald et al., 20032 | MFs, NT | MFs, AFP, Free ßhCG, uE3 | T21 | 90% | 5% |
| Wald et al., 20032 | MFs, NT | MFs, AFP, hCGß, uE3 | T21 | 90% | 5% |
| Palomaki et al., (1995)22 |  | MFs, AFP, hCGß, uE3 | T18 | 81% | 2% |
| Wald et al., 20032 | MFs, NT | MFs, AFP, Free ßhCG, uE3, inhibinA | T21 | 92% | 5% |
| Wald et al., 20032 | MFs, NT | MFs, AFP, hCGß, uE3, inhibinA | T21 | 92% | 5% |
| Chitayat et al., 2011 24 | MFs, PAPP-A | MFs, AFP, Free ßhCG, uE3, inhibinA | T21 | 85% | 4.4% |
| Chitayat et al., 2011 24 | MFs, PAPP-A | MFs, AFP, hCGß, uE3, inhibinA | T21 | 89% | 4.4% |
| Wald et al., 20032 | MFs, NT, PAPP-A | MFs, AFP, Free ßhCG, uE3 | T21 | 91% | 5% |
| Wald et al., 20032 | MFs, NT, PAPP-A | MFs, AFP, hCGß, uE3 | T21 | 91% | 5% |
| Wald et al., 20032 | MFs, NT, PAPP-A | MFs, AFP, Free ßhCG, uE3, inhibinA | T21 | 92% | 5% |
| Wald et al., 20032 | MFs, NT, PAPP-A | MFs, AFP, hCGß, uE3, inhibinA | T21 | 93% | 5% |

### Algorithm 5\_FMF\_UK: Preeclampsia screening 1st trimester

#### Description

The algorithm 5\_FMF\_UK was developed by the FMF-UK and is used for 1st trimester screening to assess the risk for the development of PE at a specified gestational age. The algorithm requires maternal factors and pregnancy dating, which can be assessed by crown-rump length (CRL), date of in vitro fertilization (IVF) or other methods. As recommended parameters Mean Arterial Blood Pressure (MAP), Uterine Artery Pulsatility Index (UAPI), PlGF and PAPP-A may be used in this algorithm. The mandatory and optional input parameters for the algorithm 5\_FMF\_UK are listed in Table 30. The algorithm requires an ultrasound scan and a blood sample for biochemical markers at 11+2 to 14+1 weeks' gestation.

The biomarkers MAP, UAPI, PlGF and PAPP-A need to be standardized as they depend on gestational age and maternal characteristics such as weight, ethnicity, and others. For standardization the FMF-UK algorithm converts the measured values into MoM values. The outputs of the software are estimated risks reported as 1:X values (Table 31).

Table 30: Input parameters for the 5\_FMF\_UK algorithm

| Input | Type | Parameter |
| --- | --- | --- |
| Mandatory |  | Patient consent |
| Mandatory |  | Identifier (e.g. patient ID) |
| Mandatory | MFs | Maternal date of birth |
| Mandatory | MFs | Height |
| Mandatory | MFs | Weight |
| Mandatory | MFs | Geographical origin |
| Mandatory | MFs | Smoking status |
| Mandatory | MFs | Diabetes status |
| Mandatory | MFs | Pregnancy dating |
| Mandatory | MFs | Method of conception |
| Mandatory | MFs | Parity |
| Mandatory if parous | MFs | Interpregnancy interval |
| Mandatory if parous | MFs | GA in last pregnancy |
| Mandatory if parous | MFs | Pre-eclampsia in previous pregnancy |
| Mandatory | MFs | Chronic hypertension |
| Mandatory | MFs | Systemic lupus erythematosus |
| Mandatory | MFs | Antiphospholipid syndrome |
| Mandatory | MFs | Preeclampsia family history |
| Mandatory | Ultrasound | Date of ultrasound examination |
| Mandatory | Ultrasound | Twin pregnancy |
| Mandatory | Ultrasound | CRL |
| Recommended | Ultrasound | MAP |
| Recommended | Ultrasound | UAPI |
| Mandatory if biochemical parameter used | Biochemical | Sample ID |
| Mandatory if biochemical parameter used | Biochemical | Sample date |
| Recommended | Biochemical | PlGF |
| Recommended | Biochemical | PAPP-A |

Table 31: Outputs of the 5\_FMF\_UK algorithm

|  |  |  |
| --- | --- | --- |
| Output | Description | Risk |
| Adjusted risk | final risk based on all the input parameters | PE < 32 WE\* |
| Adjusted risk | final risk based on all the input parameters | PE < 34 WE |
| Adjusted risk | final risk based on all the input parameters | PE < 37 WE |

\*Week (WE)

#### Analysis settings

Table 32 describes the combinations of parameters to be used for performance assessment. The performance measures of Chapter 4.5 will be computed for each combination of parameters.

Table 32: Combinations of parameters for performance assessment of the 5\_FMF\_UK algorithm

|  |  |  |  |
| --- | --- | --- | --- |
| Analysis ID | Data Type | Parameters | Adjusted risks for  PE< 32 WE, PE< 34 WE, PE< 37 WE |
| 5\_FMF\_UK\_A1 | Clinical\* | MFs, MAP, PlGF, UAPI, PAPP-A | x |
| 5\_FMF\_UK\_A2 | Clinical\* | MFs, MAP, PlGF, UAPI | x |
| 5\_FMF\_UK\_A3 | Clinical\* | MFs, MAP, PlGF, PAPP-A | x |
| 5\_FMF\_UK\_A4 | Clinical\*\* | MFs, MAP, PlGF, UAPI, PAPP-A | x |
| 5\_FMF\_UK\_A5 | Clinical\*\* | MFs, MAP, PlGF, UAPI | x |
| 5\_FMF\_UK\_A6 | Clinical\*\* | MFs, MAP, PlGF, PAPP-A | x |

\*only singleton pregnancies

\*\*only twin pregnancies

#### Validation cohorts

Table 33 lists the clinical study cohorts that are available for performance assessment. The clinical cohorts with singleton pregnancies (“T1\_PE\_FMF-UK” and “T1\_PE\_Canada”) will be combined into one clinical cohort for validation of the B·R·A·H·M·S Fast Screen PLUS algorithm.

Table 33: Clinical data for validation of the 5\_FMF\_UK algorithm

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # PE | # PE <32WE | # PE <34WE | # PE <37WE |
| T1\_PE\_FMF-UK | MFs, MAP, UAPI, PlGF, PAPP-A | 100 | 78 | 22 | 1 | 2 | 3 |
| T1\_PE\_Canada | MFs, MAP, UAPI, PlGF, PAPP-A | 5804 | 5545 | 259 | 10 | 13 | 39 |
| T1\_PE\_twins | MFs, MAP, UAPI, PlGF, PAPP-A | 1118 | 997 | 121 | 10 | 16 | 95 |

#### Acceptance criteria for validation

URP-FSP\_05\_01\_06\_01

A DR >48% will be considered as validation success for the 5\_FMF\_UK algorithm with the parameters MAP, UAPI, PlGF and PAPP-A for prediction of developing PE <32 weeks. Due to the limited availability of clinical data for pregnancies for developing PE<32 weeks (11 positive patients) a DR of 48% is used. This assures a DR point estimate above 50%. A DR >38% will be considered as validation success for the 5\_FMF\_UK algorithm with the parameters MAP, UAPI, PlGF and PAPP-A for prediction of developing PE <34 weeks. Due to the limited availability of clinical data for pregnancies for developing PE<34 weeks (15 positive patients) a DR of 38% is used. This assures a DR point estimate above 50%. A DR > 50% will be considered as validation success for the 5\_FMF\_UK algorithm with the parameters MAP, UAPI, PlGF and PAPP-A for prediction of developing PE < 37 weeks. DRs will be computed at pre-specified FPRs of 10% determined on the respective validation cohort.

For PE <32 weeks testing hypotheses are:

Null hypothesis H0: DR ≤ 48%

Alternative hypothesis H1:  DR > 48%

For PE <34 weeks testing hypotheses are:

Null hypothesis H0: DR ≤ 38%

Alternative hypothesis H1:  DR > 38%

For PE <37 weeks Testing hypotheses are:

Null hypothesis H0: DR ≤ 50%

Alternative hypothesis H1:  DR > 50%

One-sided statistical testing will be conducted by exact test for one proportion on α = 2.5% statistical significance level. Statistical power > 90% was estimated for all three endpoints: for PE <32 weeks assuming 11 available positive patients and a true DR of 90%, for PE <34 weeks assuming 15 available positive patients and a true DR of 80%, and for PE <37 weeks assuming 42 available positive patients and a true DR of 75%. Published DRs were used as the basis for the assumed true DRs (Table 34).

Table 34: References of diagnostic performance assumptions used for power calculation for the 5\_FMF\_UK algorithm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | Parameters | Indication | Fixed value | DR  (95% CI) | FPR or SPR |
| O`Gorman et al., 20178 | MFs, MAP, UAPI, PlGF, PAPP-A | PE <32 WE | FPR | 100%  (80-100) | 10% |
| Tan et al., 201825 | MFs, MAP, UAPI, PlGF, PAPP-A | PE <34 WE | SPR | 90%  (80-96) | 10% |
| O`Gorman et al., 20178 | MFs, MAP, UAPI, PlGF, PAPP-A | PE <37 WE | FPR | 80%  (67-89) | 10% |

#### Performance reporting

Point estimates and 95% confidence intervals will be reported for the performance measures described in Chapter 4.5. DR point estimates are expected to be similar to the corresponding DR estimates reported in literature (Table 35).

Table 35: Performance estimates of the 5\_FMF\_UK algorithm reported in literature

| Reference | Parameters | Indication | Fixed value | DR  (95% CI) | FPR |
| --- | --- | --- | --- | --- | --- |
| O`Gorman et al., 20178 | MFs, MAP, UAPI, PlGF, PAPP-A | PE <32 WE | FPR | 100%  (80-100) | 10% |
| O`Gorman et al., 20178 | MFs, MAP, UAPI, PlGF, PAPP-A | PE <37 WE | FPR | 80%  (67-89) | 10% |
| O`Gorman et al., 20178 | MFs, MAP, UAPI, PlGF | PE <32 WE | FPR | 100%  (80-100) | 10% |
| O`Gorman et al., 20178 | MFs, MAP, UAPI, PlGF | PE <37 WE | FPR | 75%  (62-85) | 10% |
| O`Gorman et al., 20178 | MFs, MAP, PlGF, PAPP-A | PE <32 WE | FPR | 88%  (64-99) | 10% |
| O`Gorman et al., 20178 | MFs, MAP, PlGF, PAPP-A | PE <37 WE | FPR | 69%  (56-81) | 10% |
| Tan et al., 201825 | MFs, MAP, UAPI, PlGF, PAPP-A | PE <34 WE | SPR | 90.0%  (79.5-96.2) | \*10% |
| Tan et al., 201825 | MFs, MAP, UAPI, PlGF | PE <34 WE | SPR | 90.0%  (79.5-96.2) | \*10% |
| Tan et al., 201825 | MFs, MAP, PlGF, PAPP-A | PE <34 WE | SPR | 76.7%  (64.0-86.6) | \*10% |
| Tan et al., 20189 | MFs, MAP, UAPI, PlGF, PAPP-A | PE <32 WE | Cut-off  1:100 | 94.0%  (88.1-97.1) | 14.5% |
| Tan et al., 20189 | MFs, MAP, UAPI, PlGF, PAPP-A | PE <37 WE | Cut-off  1:100 | 80.7%  (77.0-84.0) | 14.1% |
| Tan et al., 20189 | MFs, MAP, UAPI, PlGF | PE <32 WE | Cut-off  1:100 | 94.0%  (88.1-97.1) | 14.5% |
| Tan et al., 20189 | MFs, MAP, UAPI, PlGF | PE <37 WE | Cut-off  1:100 | 80.7%  (77.0-84.0) | 14.1% |
| Tan et al., 20189 | MFs, MAP, PlGF, PAPP-A | PE <32 WE | Cut-off  1:100 | 87.9%  (80.8-92.7) | 15.1% |
| Tan et al., 20189 | MFs, MAP, PlGF, PAPP-A | PE <37 WE | Cut-off  1:100 | 77.5%  (73.6-81.0) | 14.7% |

\*SPR

### Algorithm 6\_FMF\_UK: Preeclampsia screening 2nd trimester

#### Description

The algorithm 6\_FMF\_UK was developed by the FMF-UK and is used for 2nd trimester screening to assess the risk for the development of PE at a specified gestational age for singleton pregnancy. The algorithm requires maternal factors and pregnancy dating, which can be assessed by crown-rump length (CRL), date of in vitro fertilization (IVF) or other methods. Recommended parameters for this algorithm are MAP, UAPI, PlGF and sFlt-1. The mandatory and recommended input parameters for the algorithm 6\_FMF\_UK are listed in Table 36. The algorithm requires an ultrasound scan and a blood sample for biochemical markers at 19+0 to 24+6 weeks' gestation.

The biomarkers MAP, UAPI, PlGF and sFlt-1 need to be standardized as they depend on gestational age and maternal characteristics such as weight, ethnicity, and others. For standardization the FMF-UK algorithm converts the measured values into so-called “Multiples of Median” (MoM) values. The outputs of the software are estimated risks reported as a 1:X values (Table 37).

Table 36: Input parameters for the 6\_FMF\_UK algorithm

|  |  |  |
| --- | --- | --- |
| Input | Type | Parameters |
| Mandatory |  | Patient consent |
| Mandatory |  | Identifier (e.g. patient ID) |
| Mandatory | MFs | Maternal date of birth |
| Mandatory | MFs | Height |
| Mandatory | MFs | Weight |
| Mandatory | MFs | Geographical origin |
| Mandatory | MFs | Smoking status |
| Mandatory | MFs | Diabetes status |
| Mandatory | MFs | Pregnancy dating |
| Mandatory | MFs | Method of conception |
| Mandatory | MFs | Parity |
| Mandatory if parous | MFs | Interpregnancy interval |
| Mandatory if parous | MFs | GA in last pregnancy |
| Mandatory if parous | MFs | Pre-eclampsia in previous pregnancy |
| Mandatory | MFs | Chronic hypertension |
| Mandatory | MFs | Systemic lupus erythematosus |
| Mandatory | MFs | Antiphospholipid syndrome |
| Mandatory | MFs | Preeclampsia family history |
| Mandatory if ultrasound parameter used | Ultrasound | Date of ultrasound examination |
| Mandatory | Ultrasound | Singelton pregnancy |
| Mandatory if pregnancy dating is CRL | Ultrasound | CRL |
| Recommended | Ultrasound | MAP |
| Recommended | Ultrasound | UAPI |
| Mandatory if biochemical parameter used | Biochemical | Sample ID |
| Mandatory if biochemical parameter used | Biochemical | Sample date |
| Recommended | Biochemical | PlGF |
| Recommended | Biochemical | sFlt-1 |

Table 37: Outputs of the algorithm 6\_FMF\_UK

|  |  |  |
| --- | --- | --- |
| Output | Description | Risk |
| Adjusted risk | Final risk based on all the input parameters | PE < 32 WE |
| Adjusted risk | Final risk based on all the input parameters | PE < 37 WE |

#### Analysis settings

Table 38 summarizes the combinations of parameters to be used for performance. The performance measures of Chapter 4.5 will be computed for each combination of parameters.

Table 38: Combinations of parameters for performance assessment of the 6\_FMF\_UK algorithm

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Analysis ID | Data Type | Parameters | Adjusted risk PE< 32 WE | Adjusted risk PE< 37 WE |
| 6\_FMF\_UK\_A1 | Clinical | MFs, MAP, PlGF, sFlt-1, UAPI | x | x |
| 6\_FMF\_UK\_A2 | Clinical | MFs, MAP, PlGF, sFlt-1 | x | x |

#### Validation cohorts

The validation cohort used for validation of the 6\_FMF\_UK algorithm is listed in Table 39.

Table 39: Clinical data for validation of the 6\_FMF\_UK algorithm

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # PE | # PE  <32 WE | # PE  <37 WE |
| T2\_PE\_FMF-UK | MFs, MAP, UAPI, PlGF, sFlt-1 | 1000 | 900 | 100 | 7 | 24 |

#### Acceptance criteria for validation

URP-FSP\_05\_01\_07\_01

A DR > 50% will be considered as validation success for the 6\_FMF\_UK algorithm with the parameters MAP, UAPI, PlGF and sFlt-1 for prediction of developing PE <37 weeks. The DR will be computed at pre-specified FPR of 10% determined on the respective validation cohort.

For PE <37 weeks testing hypotheses are:

Null hypothesis H0: DR ≤ 50%

Alternative hypothesis H1:  DR > 50%

One-sided statistical testing will be conducted by exact test for one proportion on α = 2.5% statistical significance level. Statistical power > 90% was estimated for PE <37 weeks assuming 24 available positive patients and a true DR of 83%. Published DRs were used as the basis for the assumed true DRs (Table 40).

Due to the limited availability of clinical data for pregnancies developing PE<32 weeks one-sided statistical testing is regarded not adequate for this endpoint (only 7 affected pregnancies are available, this would result in insufficiently informative testing). Accordingly, validation success will already be considered if the DR for the 7 pregnancies developing PE <32 weeks according to adjusted risks is larger than or equal to the DR according to corresponding prior risks at a fixed FPR of 1%.

Table 40: References of diagnostic performance assumptions used for power calculation for the 6\_FMF\_UK algorithm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | Parameters | Indication | Cut-off | DR  (95% CI) | SPR |
| Litwinska et al., 201810 | MFs, MAP, UAPI, PlGF, sFlt-1 | PE <32 WE | 1:25 | 100%  (85.2-100) | 0.8% |
| Litwinska et al., 201810 | MFs, MAP, UAPI, PlGF, sFlt-1 | PE between 32+0 and 35+6 WE | 1:150 | 89.7%  (78.8-96.1) | 9.1% |

#### Performance reporting

URP-FSP\_05\_01\_07\_01

Point estimates and 95% confidence intervals will be reported for the performance measures described in Chapter 4.5. DR point estimates are expected to be similar to the corresponding DR estimates reported in literature (Table 41).

Table 41: Performance estimates of the 6\_FMF\_UK algorithm reported in literature

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | Parameters | Indication | Cut-off | DR  (95% CI) | SPR |
| Litwinska et al., 201810 | MFs, MAP, UAPI, PlGF, sFlt-1 | PE <32 WE | 1:25 | 100%  (85.2-100) | 0.8% |
| Litwinska et al., 201810 | MFs, MAP, UAPI, PlGF, sFlt-1 | PE between 32+0 and 35+6 WE | 1:150 | 89.7%  (78.8-96.1) | 9.1% |
| Litwinska et al., 201810 | MFs, MAP, PlGF, sFlt-1 | PE <32 WE | 1:25 | 87.0%  (66.4-97.2) | 0.6% |
| Litwinska et al., 201810 | MFs, MAP, PlGF, sFlt-1 | PE between 32+0 and 35+6 WE | 1:150 | 75.9%  (62.8-86.1) | 10.3% |

### Algorithm 7\_FMF\_UK: Preeclampsia screening 3rd trimester

#### Description

The algorithm 7\_FMF\_UK was developed by the FMF-UK and is used for 3rd trimester screening to assess the risk for the development of PE at a specified gestational age for singleton pregnancy. The algorithm requires maternal factors and pregnancy dating, which can be assessed by crown-rump length (CRL), date of in vitro fertilization (IVF) or other methods. Recommended parameters for this algorithm are MAP, UAPI, PlGF and sFlt-1. The mandatory and recommended input parameters for the algorithm 7\_FMF\_UK are listed in Table 42. The algorithm requires an ultrasound scan and a blood sample for biochemical markers at 30+0 to 37+6 weeks' gestation.

The biomarkers MAP, UAPI, PlGF and sFlt-1 need to be standardized as they depend on gestational age and maternal characteristics such as weight, ethnicity, and others. For standardization the FMF-UK algorithm converts the measured values into so-called “Multiples of Median” (MoM) values. The outputs of the software are estimated risks reported as a 1:X values and the adjusted risk of developing PE at a specified gestational age is reported according to gestational age at screening (Table 43).

Table 42: Input parameters for the 7\_FMF\_UK algorithm

| Input | Type | Parameters |
| --- | --- | --- |
| Mandatory |  | Patient consent |
| Mandatory |  | Identifier (e.g. patient ID) |
| Mandatory | MFs | Maternal date of birth |
| Mandatory | MFs | Height |
| Mandatory | MFs | Weight |
| Mandatory | MFs | Geographical origin |
| Mandatory | MFs | Smoking status |
| Mandatory | MFs | Diabetes status |
| Mandatory | MFs | Pregnancy dating |
| Mandatory | MFs | Method of conception |
| Mandatory | MFs | Parity |
| Mandatory if parous | MFs | Interpregnancy interval |
| Mandatory if parous | MFs | GA in last pregnancy |
| Mandatory if parous | MFs | Pre-eclampsia in previous pregnancy |
| Mandatory | MFs | Chronic hypertension |
| Mandatory | MFs | Systemic lupus erythematosus |
| Mandatory | MFs | Antiphospholipid syndrome |
| Mandatory | MFs | Preeclampsia family history |
| Mandatory if ultrasound parameter used | Ultrasound | Date of ultrasound examination |
| Mandatory | Ultrasound | Singelton pregnancy |
| Mandatory if pregnancy dating is CRL | Ultrasound | CRL |
| Recommended | Ultrasound | MAP |
| Recommended | Ultrasound | UAPI |
| Mandatory if biochemical parameter used | Biochemical | Sample ID |
| Mandatory if biochemical parameter used | Biochemical | Sample date |
| Recommended | Biochemical | PlGF |
| Recommended | Biochemical | sFlt-1 |

Table 43: Outputs of the 7\_FMF\_UK algorithm

|  |  |  |  |
| --- | --- | --- | --- |
| Output | Description | Screening at GA | Risk |
| Adjusted risk | final risk based on all the input parameters | 30+0 to 34+6 WE | PE< 37 WE  PE< 42 WE |
| Adjusted risk | final risk based on all the input parameters | 35+0 to 37+6 WE | PE< 42 WE |

#### Analysis settings

Table 44 summarizes the combinations of parameters to be used for performance. The performance measures of Chapter 4.5 will be computed for each combination of parameters.

Table 44: Combinations of parameters for performance assessment of the 7\_FMF\_UK algorithm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Analysis ID | Data Type | Parameters | Screening at GA | Adjusted risk  PE< 37 WE | Adjusted risk  PE< 42 WE |
| 7\_FMF\_UK\_A1 | Clinical | MFs, MAP, PlGF, sFlt-1, UAPI | 30+0 to 34+6 WE | x | x |
| 7\_FMF\_UK\_A2 | Clinical | MFs, MAP, PlGF, sFlt-1, UAPI | 35+0 to 37+6 WE | - | x |
| 7\_FMF\_UK\_A3 | Clinical | MFs, MAP, PlGF, sFlt-1 | 30+0 to 34+6 WE | x | x |
| 7\_FMF\_UK\_A4 | Clinical | MFs, MAP, PlGF, sFlt-1 | 35+0 to 37+6 WE | - | x |

#### Validation cohorts

The validation cohorts used for validation of 7\_FMF\_UK are described in Table 45.

Table 45: Clinical data for validation of the 7\_FMF\_UK algorithm

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cohort ID | Parameters | Total | # Controls | # PE | # PE <37WE | # PE <42WE |
| T3\_PE\_30\_34\_Canada | MFs, MAP, UAPI, PlGF, sFlt-1 | 782 | 751 | 31 | 7 | 31 |
| T3\_PE\_35\_37\_FMF\_UK | MFs, MAP, UAPI, PlGF, sFlt-1 | 1000 | 900 | 100 | 8 | 97 |

#### Acceptance criteria for validation

URP-FSP\_05\_01\_07\_01

A DR >35% will be considered as validation success for the 7\_FMF\_UK algorithm with the parameters MAP, UAPI, PlGF and sFlt-1 for prediction of developing PE <42 weeks by screening at GA of 30+0 to 34+6 weeks. Due to the limited availability of clinical data for pregnancies for developing PE<42 weeks (31 positive patients screened at GA of 30+0 to 34+6 weeks) a DR of 35% is used. This assures a DR point estimate above 50%. A DR > 50% will be considered as validation success for the 7\_FMF\_UK algorithm with the parameters MAP, UAPI, PlGF and sFlt-1 for prediction of developing PE <42 weeks by screening at GA of 35+0 to 37+6 weeks. DRs will be computed at a pre-specified FPR of 10% determined on the respective validation cohort.

For PE <42 weeks screened at GA of 30+0 to 34+6 testing hypotheses are:

Null hypothesis H0: DR ≤ 35%

Alternative hypothesis H1:  DR > 35%

For PE <42 weeks screened at GA of 34+0 to 37+6 testing hypotheses are:

Null hypothesis H0: DR ≤ 50%

Alternative hypothesis H1:  DR > 50%

One-sided statistical testing will be conducted by exact test for one proportion on α = 2.5% statistical significance level. Statistical power > 90% estimated for both testing scenarios based on available sample sizes and assumed true performances taken from literature (Table 46).

* DR of screening at GA of 30+0 to 34+6 weeks: 31 cases, 70% assumed true DR
* DR of screening at GA of 34+0 to 37+6 weeks: 97 cases, 70% assumed true DR

Due to the limited availability of clinical data for pregnancies screened at GA of 30+0 to 34+6 weeks for developing PE<37 weeks one-sided statistical testing is regarded not adequate for this endpoint (only 7 affected pregnancies are available, this would result in insufficiently informative testing). Accordingly, validation success will already be considered if the DR for the 7 pregnancies developing PE <37 weeks according to adjusted risks is larger than or equal to the DR according to the corresponding prior risks at a fixed FPR of 10%.

Table 46: References of diagnostic performance assumptions used for power calculation for the 7\_FMF\_UK algorithm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | Parameters | Indication | Fixed | DR  (95% CI) | FPR |
| Tsiakkas et al., 2016 12 | MFs, MAP, UAPI, PlGF, sFlt-1 | PE <37 WE | FPR | 98%  (88-99) | 10% |
| Andrietti et al., 201613 | MFs, MAP, UAPI, PlGF, sFlt-1 | PE ≥37 WE | FPR | 82%  (70-91) | 10% |
| Tsiakkas et al., 2016 12 | MFs, MAP, UAPI, PlGF, sFlt-1 | PE ≥37 WE | FPR | 65%  (58-72) | 10% |

#### Performance reporting

URP-FSP\_05\_01\_07\_01

Point estimates and 95% confidence intervals will be reported for the performance measures described in Chapter 4.5. DR point estimates are expected to be similar to the corresponding DR estimates reported in literature (Table 47).

Table 47: Performance estimates of the 7\_FMF\_UK algorithm reported in literature

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Reference | Screening at GA | Parameters | Indication | Fixed | DR  (95% CI) | FPR |
| Tsiakkas et al., 201612 | 30+0 to 34+6 WE | MFs, MAP, UAPI, PlGF, sFlt-1 | PE <37 WE | FPR | 98%  (88-99) | 10% |
| Tsiakkas et al., 201612 | 30+0 to 34+6 WE | MFs, MAP, UAPI, PlGF, sFlt-1 | PE ≥37 WE | FPR | 65%  (58-72) | 10% |
| Tsiakkas et al., 201612 | 30+0 to 34+6 WE | MFs, MAP, PlGF, sFlt-1 | PE <37 WE | FPR | 98%  (88-99) | 10% |
| Tsiakkas et al., 201612 | 30+0 to 34+6 WE | MFs, MAP, PlGF, sFlt-1 | PE ≥37 WE | FPR | 66%  (59-73) | 10% |
| Andrietti et al., 201613 | 35+0 to 37+6 WE | MFs, MAP, UAPI, PlGF, sFlt-1 | PE ≥37 WE | FPR | 82%  (70-91) | 10% |
| Andrietti et al., 201613 | 35+0 to 37+6 WE | MFs, MAP, PlGF, sFlt-1 | PE ≥37 WE | FPR | 77%  (65-87) | 10% |
| Panaitescu et al., 201811 | 35+0 to 36+6 WE | MFs, MAP, UAPI, PlGF, sFlt-1 | PE ≥37 WE | Cut-off 1:20 | 68.8%  (62.9-74.2) | 9.1% |
| Panaitescu et al., 201811 | 35+0 to 36+6 WE | MFs, MAP, PlGF, sFlt-1 | PE ≥37 WE | Cut-off 1:20 | 70.2%  (64.4-75.6) | 9.7% |

# Accompanying Documents

| **Document Number** | **Document Title** |
| --- | --- |
| HN-EXT-2204 R03 | IVDR 2017/746 Regulation for in vitro diagnostic |
| HN-EXT-2284 R01 | MDCG 2020-1 Guidance on Clinical Evaluation (MDR) / Performance Evaluation (IVDR) of Medical Device Software |
|  | CLSI I\LA25 A2:2011: Maternal Serum Screening |
| HN-EXT-0772 R04 | CLSI EP12-Ed3: 2023: Evaluation of Qualitative, Binary Output Examination Performance |
| HN-EXT-1953 R01 | ISO 20916:2019 (E) In vitro diagnostic medical devices-Clinical performance studies using specimens from human subjects-Good study practice |
| As referenced in HN-EXT-0166 R35 | GHTF/SG5/N6:2012: Clinical Evidence for IVD medical devices – Key Definitions and Concepts |
| As referenced in HN-EXT-0166 R35 | GHTF/SG5/N7:2012: Clinical Evidence for IVD medical devices - Scientific Validity Determination and Performance Evaluation |
| As referenced in HN-EXT-0166 R35 | GHTF/SG5/N8:2012: Clinical Evidence for IVD Medical Devices - Clinical Performance Studies for In Vitro Diagnostic Medical Devices |
| As referenced in HN-EXT-0166 R35 | ISO 3534-1: Statistics Vocabulary and symbols Part 1: General statistical terms and terms used in probability |
| As referenced in HN-EXT-0166 R35 | ISO 3534-2: Statistics Vocabulary and symbols Part 2: Applied statistics |
| As referenced in HN-EXT-0166 R35 | ISO 3534-3: Statistics Vocabulary and symbols Part 3: Design of experiments |
| HN-SOP-0022 R09 | Performance Evaluation for IVD Products |
| HN-SOP-0019 R08 | Clinical Trial Control |
| HN-SOP-0094 R17 | Design Control |
| HN-SOP-0018 R08 | Application of Statistical Methods to Generate Data-Based Evidence |
| HN-PD-7013 R05 | URP B·R·A·H·M·S Fast Screen PLUS 1.0 software |
| HN-PD-5805 R03 | Intended Purpose Intended Use Statement BRAHMS Fast Screen Plus (IVDR) |
| HN-PD-10006 R01 | Performance Evaluation Plan B·R·A·H·M·S Fast Screen PLUS |

# Terms and Abbreviations

| **Terms / Abbreviations** | **Explanation** |
| --- | --- |
| AFP | Alpha Fetoprotein |
| CE | Conformité Européenne |
| CLSI | Clinical Laboratory Standards Institute |
| CRL | Crown-rump Length |
| DHF | Design History File |
| DOE | Degrees of Extremeness |
| DR | Detection rate |
| DV | Ductus Venosus Flow |
| DVPI | Ductus Venosus Pulsatility Index |
| FDA | Food and Drug Administration |
| FHR | Fetal Heart Rate |
| FMF-DE | Fetal Medicine Foundation Germany |
| FMF-UK | Fetal Medicine Foundation United Kingdom |
| FPR | False Positive Rate |
| Free βhCG | Free β human Chorionic Gonadotropin |
| GA | Gestational Age |
| GHTF | Global Harmonization Task Force |
| GMkt | Global Marketing |
| GMA | Global Medical Affairs |
| hCGß | Human Chorionic Gonadotropin hormone and its Free beta subunit |
| ID | Identifier |
| IPIUS | Intended Purpose/ Intended Use Statement |
| ISO | International Standards Organization |
| IVD | In Vitro Diagnostic |
| IVDR | In Vitro Diagnostic Medical Devices Regulation |
| IVF | In Vitro Fertilization |
| LER | Literature Evaluation Report |
| LIS | Laboratory Information System |
| LSR | Literature Search Report |
| MDCG | Medical Devices Coordination Group |
| MAP | Mean Arterial Blood Pressure |
| MDSW | Medical Device Software |
| MFs | Maternal Factors |
| MoM | Multiples of Median |
| NPV | Negative Predictive Value |
| NTD | Neural Tube Defect |
| PAPP-A | Pregnancy Associated Plasma Protein A |
| PE | Pre-eclampsia |
| PEP | Performance Evaluation Plan |
| PER | Performance Evaluation Report |
| PlGF | Placental Growth Factor |
| PPV | Positive Predictive Value |
| NB | Nasal Bone |
| NT | Nuchal Translucency |
| NTD | Neural Tube Defect |
| R&D | Research and Development |
| RAC | Risk Assessment, Clinical |
| RAT | Risk Assessment, Technical |
| RMR | Risk Management Report |
| sFlt-1 | Soluble FMS like tyrosine kinase-1 |
| SOP | Standard Operating Procedure |
| SPR | Screen Positive Rate |
| SVCPR | Scientific Validity and Clinical Performance Report |
| T21 | Trisomy 21 |
| T18 | Trisomy 18 |
| T13 | Trisomy 13 |
| UAPI | Uterine Artery Pulsatility Index |
| uE3 | Unconjugated estriol |
| TR | Tricuspid Regurgitation |
| TS | Technical Specifications |
| uE3 | Unconjugated Estradiol 3 |
| URP | User Requirement Profile |
| VSR | Verification Summary Report |
| WE | Weeks |
| WHO | World Health Organization |

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# Revision history

|  |  |  |  |
| --- | --- | --- | --- |
| **Revision History** | | | |
| Version No. | Description of change | Author | Date (MM/YYYY) |
| R01 | Initial version of CPSP | Dr. Heike Göhler (GMA) | 02/2024 |