

Protocole SAFE 21

Projet de Soutien aux Techniques Innovantes et Coûteuses (STIC)
ADVANCED NON INVASIVE SCREENING FOR TRISOMY 21
FROM MATERNAL BLOOD
SAFE 21 STUDY
Version N°5 : 24/11/2015

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PROTOCOL SIGNATURE PAGE

Research code : P130601

Title : Dépistage avancé non invasif de la trisomie 21 sur sang maternel. Étude SAFE 21

Version n°5 : 24 Novembre 2015

(Advanced non invasive screening for trisomy 21 from maternal blood. SAFE 21 study)

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This protocol received a favorable opinion from the committee for the protection of people (Ethical Approval), Ile De France XI St Germain en Laye

STUDY SYNOPSIS

<i>Full Title</i>	Advanced non invasive screening for trisomy 21 from maternal blood.
<i>Acronym</i>	<i>SAFE 21 Study</i>
<i>Principal Investigator</i>	<i>Prof Laurent Salomon</i>
<i>Sponsor</i>	<i>Assistance Publique – Hôpitaux de Paris</i>
<i>General Objectives</i>	<p><i>The general objectives are :</i></p> <ul style="list-style-type: none"> <i>-To facilitate the fast and harmonious distribution of the techniques of non-invasive prenatal testing(NIPT) based on the analysis of circulating Fetal DNA and answer the needs expressed by the women and healthcare professionals, to improve the care of the women at high risk for trisomy 21 following 1st trimester combined screening and to reduce the number of invasive tests and inferred miscarriages.</i> <i>- To evaluate the introduction of NIPT into routine medical care (reduction of the rate of invasive tests and complications of these procedures), confirmation of the diagnostic performances and the feasibility of the biological medical analysis (acceptability in practice and economically) in comparison with the classic prenatal diagnosis.</i> <i>- To specify how to best implement NIPT in the national organization of prenatal screening for trisomy 21 (whether or not to subsequently readjust the thresholds of the tests and their combination).</i> <i>- To promote the structuring and organization of professional networks concerned with the prenatal screening of the trisomy 21, to allow the emergence of a global system of collection of outcomes thus improving the</i>

	<p><i>quality of the general practice. The reserved criteria of evaluation are:</i></p> <ul style="list-style-type: none"> <i>· Primary:</i> <ul style="list-style-type: none"> <i>- percentage of Fetal losses in each group</i> <i>· Secondary:</i> <ul style="list-style-type: none"> <i>- percentage of invasive testing in each group (amniocentesis or chorionic villous sampling)</i> <i>- diagnostic performances of NIPT, in particular the false positive and negative rate</i> <i>- percentage of obtaining the NIPT result within the time limits allowed and average time taken for obtaining a NIPT result</i> <i>- percentage failed or inconclusive NIPT results</i> <i>- percentage of other discovered anomalies found with invasive testing</i> <i>- percentage of invasive tests performed in spite of a reassuring NIPT result (ie for anomaly or reinsurance)</i> <i>- association between the maternal characteristics (weight, height, parity, history, serum markers) and the NIPT result</i> <i>- costing of NIPT in clinical practice, and the budgetary impact of its use compared with the conventional screening.</i>
<i>Experimental design</i>	<i>It is an open-labelled multicenter randomized control study involving a technique previously validated in the literature and within the framework of a PHRC (Seq 21).</i>

	<p><i>This study aims to evaluate and promote in clinical practice, the NIPT based on Fetal DNA circulating in the maternal blood. The randomization will be stratified by center.</i></p> <p><i>The duration of participation in the study will be 7 months maximum for each patient included in the protocol. The duration of the inclusions will be 24 months.</i></p> <p><i>A decision will be taken according to the allocated group.</i></p>
<i>Target Population</i>	<p><i>Pregnant women with a high risk of trisomy 21 estimated on the basis of the combined or sequential integrated screening (risk between 1/5 and 1/250).</i></p>
<i>Inclusion criteria</i>	<p><i>The following patients will be included in the protocol:</i></p> <ul style="list-style-type: none"> <i>- 18 years old or over,</i> <i>- Singleton pregnancy,</i> <i>- A high risk of trisomy 21 estimated on the basis of the combined or sequential integrated screening (risk between 1/5 and 1/250),</i> <i>- At a gestational age between 11 and 18 weeks</i> <i>- Those wishing to have an invasive prenatal diagnosis,</i> <i>- Agree to be contacted again by questionnaire after the birth</i> <i>- Accept a karyotype if NIPT + or if allocated in the invasive group</i> <i>- In possession of a social security number.</i> <i>- Accepts and signs the informed consent.</i>

<i>Exclusion criteria</i>	<p><i>The following patients will not be included in the study:</i></p> <ul style="list-style-type: none"> - <i>A combined risk of < 1/250 or > 1/5,</i> - <i>A nuchal translucency > 3 mm, and/or a measurement of PAPP-A and/or of BhCG <0.3 MoM or > 5 MoM.</i> - <i>Fetal anomaly diagnosed or suspected at the first trimester ultrasound,</i> - <i>One of the parents being a carrier of a balanced chromosomal translocation (involving chromosome 21 or not)</i> - <i>Not wishing a priori invasive prenatal diagnosis of chromosomal abnormalities,</i> - <i>Concurrent participation in another trial,</i> - <i>Possibility of a "vanishing twin "</i> - <i>Poor understanding of the French language</i> - <i>Or refusing to sign the informed consent.</i>
<i>Acts added by the research</i>	<i>Maternal blood sample of 20ml</i>
<i>Risks added by the research</i>	<i>A</i>
<i>Number of participants' necessary</i>	<i>2450</i>
<i>Number of participating centers</i>	<i>69</i>
<i>Duration of the study</i>	<ul style="list-style-type: none"> - <i>Duration of inclusion: 30 months</i> - <i>Duration of participation: 7 months</i> - <i>Total duration :37 months</i>
<i>Number of planned participants per center per month</i>	<i>7 to 8</i>
<i>Source of funding</i>	<i>STIC 2012</i>

<i>Independent supervisory committee</i>	<i>Given the nature of the tested procedure, the constitution of a supervisory committee of severe adverse events is not required</i>
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1. INTRODUCTION:

Current situation:

Trisomy 21 is the most frequent cause of disability of chromosomal origin with an approximate prevalence of 1.3/1000, and this increases with advancing maternal age¹. Several methods of prenatal screening have been developed. In France, the High Authority of Health (Haute Autorité de Santé - HAS) recommended (2007) that 1st trimester combined screening between 11+ 0 and 13+ 6 weeks' gestation, is proposed to all the women. This screening is based on the combination of the maternal age, the measure of the crown rump length (CRL), the nuchal translucency and the serum markers². This screening program has been effective in France since June 23rd, 2009 establishing the practice guidelines for screening and diagnosis of trisomy 21 based on maternal serum markers (Journal Officiel of July 03rd, 2009)³. Approximately 85 % of women in France avail of this screening. The screening tests are performed following a detailed explanation of the process and informed patient consent. The sensitivity of these screening tests is about 85 % for a false positive rate of 5 %⁴⁻⁶.

Following screening, a risk of $> 1/250$, which is considered a screen positive result (risk adjusted according to age, and the measure of the nuchal translucency and the serum markers), occurs in just under 5 % of the women⁷, a diagnostic test is then offered and performed in the majority of these high-risk women. This test consists of an invasive procedure by taking a sample of chorionic villi (CVS), performed during the screening period at 11-14 weeks, or by amniocentesis, which is performed after 15 weeks' gestation. However, the positive predictive value (PPV) of this combined screening method remains low, of the order of 1/30, which means that a diagnosis of trisomy 21 occurs only in 1 case for every 30 invasive procedures^{4-6,8}. In addition to maternal anxiety, these invasive diagnostic tests are associated with a risk of fetal loss of approximately 1/200 in 1/100^{9,10}, a risk of maternal morbidity^{11,12} and other important costs such as the test itself, hospital admissions, and sick leave from work.

In France, would the entire population of pregnant women perform this screening test, about 40,000 would screen positive, meaning 40,000 invasive procedures would be proposed, to diagnose less than 2000 cases of trisomy 21⁷. The positive predictive value of the combined screening in France is comparable to that quoted in the literature, therefore these procedures could be responsible for 200 to 400 fetal losses due to miscarriages attributable to the invasive procedure and would also contribute to maternal morbidity and a significant public health cost.

Non-invasive approach to prenatal screening:

Approximately 10 % of the circulating free DNA, which can be collected during the first trimester of pregnancy in the maternal plasma, is of fetal origin and this DNA is specific to the current pregnancy¹³⁻¹⁷. This opened the way for the possibility of a non-invasive method of prenatal testing/screening (NIPT) of the fetal chromosomes, as direct access to the fetal material in the maternal blood was achievable. These methods improved dramatically during the past 10 years and this approach, appears to be more efficient than the approaches based on RNA or fetal cells. The sequencing of all the DNA circulating in the maternal blood facilitates the analysis of the quantity of DNA originating from every chromosome and also prevent from differentiating between the maternal and fetal DNA. This fetal DNA circulating in the maternal blood can be used to aid in the diagnosis of the most current aneuploidies, including the trisomy 21, as well as trisomy 18 and 13^{16,21-34}. Several recent studies in populations of women at high risk and even those at intermediate risk showed that an analysis of the cell free fetal DNA circulating in the maternal blood is possible and allows to detect a fetal trisomy 21 with a sensitivity of more than 98 % and a false positive rate of 3 % (Table).

<i>Author (date)</i>	<i>N</i>	<i>N Trisomies</i>	<i>Type Trisomy</i>	<i>Se/Sp T21</i>	<i>Se/Sp T18</i>	<i>Se/Sp T13</i>	<i>Doute</i>	<i>Type of NIPT</i>	<i>Population</i>
<i>Palomaki 2011 Palomaki 2012</i>	1971	283 212 (T21) 59 (T18) 12(T13)	21 18 13	98.6/99.8	100 / 972	91.7 / 99.03	1,5%	MPSS	High risk
<i>Sehnert 2011</i>	119	53	21 18	100 /?	100 / ?	-	?	MPSS	High risk
<i>Ehrich 2011</i>	480	39	21	100	99.7	-	4,8%	MPSS	High risk
<i>Bianchi 2012</i>	532	221	21 18 13	100/?	97.2 / ?	78.6/	5.8%	MPSS	High risk
<i>Chiu 2011</i>	753	86	21	100/97.9	-	-		MPSS	High risk
<i>Ashoor 2012</i>	400	100	21 18	100/99	100 / 99		6.6%	DANSR	Serum linrary
<i>Sparks 2012</i>	338	88	21 18	100/?	100 / ?		?	DANSR	High risk
<i>Norton 2012</i>	3228	119 81(T21) 38(T18)	21 18	100 / (95.5- 100)	97 (86.5- 99.9)		4.6%	DANSR	High risk
<i>SEQ 21</i>	145	31 (T21)	21	100/100			2,7%	MPSS	High risk

This approach adds a considerable improvement to screening and there is good evidence of its reliability, robustness and reproducibility, which can be adapted and offered to a population undergoing the combined screening program ^{21,24,26,29,35}, to drastically decrease the risk of trisomy 21, thus giving woman with a screen positive result an alternative to an invasive test which has a risk of fetal loss. The scientific evidence for this from various media sources (" non invasive screening trisomy 21 ", 17000 Google results on 15/11/2012), justifies the considerable demand of the practitioners and women for its implementation.

The Genoscope (Evry-Prof Weissenbach) and Cytogenetics teams of the hospital Necker-Enfants Malades (Prof Vekemans), associated with the maternity departments of the hospital Necker-Enfants Malades (Prof Ville), Robert Debré (Prof Oury) and Poissy (Prof Fauconnier) demonstrated the feasibility and the validity of these techniques in a population of women who screened high risk for trisomy 21. Within the framework of this Hospital Project of Clinical Research (PHRC) Seq 21 (PHRC on 2009 – Principal Investigator Prof Laurent Salomon, Scientific Director Prof Vekemans), it was possible to blindly correctly identify 150 controls and 31 cases of trisomy 21, without any false positives or negatives ³⁶. The final investigations of this project will be performed in the next few weeks. In a similar manner as reported in the literature, we had collected the maternal blood before any invasive procedure in women at high risk of fetal trisomy 21 ($> 1/250$). In 5 cases, however, the test failed (2.8 % of the cases) which is in line with the reported data ^{17-19,21,24,26,29-32,37}.

This PHRC essentially aimed to test the reproducibility feasibility of this widely published and validated technique ^{17-19,21,24,26,29-32,37}. The techniques were developed and mastered by the medical staff and the cytogeneticists at Hospital Necker-Enfants Malades.

The results obtained from the PHRC Seq 21 study ³⁶, as well as those from the numerous other published studies validating these techniques of sequencing, justify offering this type of test, to women at high risk of trisomy 21, after a first trimester combined screening test. It would drastically decrease their risk (by a factor of between 50 and infinity ^{19,22,24,26,29-31,36,37}) thus allowing women at risk to avoid invasive testing. However, the full health economic impact of NIPT has not yet been demonstrated. If NIPT was widely implemented, would this approach really decrease the quoted miscarriage rate following invasive testing, rates that have been derived from one single study ⁹ and have been subsequently questioned ^{38,39}? Is there a risk of

not obtaining a conventional karyotype in women at high risk, as karyotyping can sometimes discover unexpected anomalies or mosaic forms of chromosomal abnormalities⁴⁰⁻⁴⁵? Could this reduce the anxiety of the women^{46,47}? Is the model economically viable? The answers to these questions can only be addressed by a randomized study with the objective of estimating the clinical and medical economic utility of the implementation of NIPT in the current screening program, prior to its widespread introduction.

2. OBJECTIVES

This project of Support for the Innovative and expensive Techniques (STIC) entitled "SAFE 21" continues the work of the PHRC Seq 21³⁶.

The general objectives within the framework of the STIC study are:

- Estimation of the impact of introducing NIPT into medical practice (reduction of the rate of invasive procedures and their complications, confirmation of the diagnostic performances and the feasibility, acceptability in practice) and its economic impact in comparison with the standard prenatal diagnosis.
- Facilitate the rapid and harmonious implementation of Non-Invasive Prenatal Testing/Screening (NIPT) based on the analysis of the circulating cell free fetal DNA and to improve the management of women at high risk following the first trimester combined screening and to reduce the number of invasive procedures and associated miscarriages.

- To specify how to best implement NIPT in the national organization of prenatal screening for trisomy 21 (whether or not to subsequently readjust the thresholds of the tests and their combination).
- Promote the structuring and the organization of professional networks for the introduction of prenatal screening for trisomy 21, and to allow the emergence of a global system of collection of outcomes facilitating the improvement of the quality of practice.

In practice, the main objective is the reduction in the rate of invasive tests and their complications. The main criteria of evaluation will be:

Primary:

- Percentage of fetal losses in every group

Secondary:

- Percentage of invasive tests in each group (amniocentesis, chorionic villous sampling, or fetal blood sampling)
- Diagnostic Performances of the NIPT, in particular the false positive and negative rates
- Percentage of cases in which the result of the NIPT was obtained within the time limits allowed and average time taken to obtain the results
- Percentage of inconclusive results or failure of the NIPT
- Percentage of other additional anomalies discovered by invasive testing
- Percentage of invasive tests performed in spite of a reassuring NIPT result
- Association between the maternal characteristics (weight, height, parity, history, serum markers) and the results of the NIPT
- Costing of the NIPT in clinical practice, and the monetary impact of its use with regard to the conventional screening.

3. EXPERIMENTAL PLAN

This is a randomized controlled multi-center open study involving a technique previously validated in the literature and within the framework of the PHRC (Seq 21). This study is concerned with the implementation of NIPT into clinical practice, a technique based on the analysis of the cell free fetal DNA circulating in the maternal blood. The randomization of each patient will be conducted by each participating center.

The duration of participation in the study for each patient will be 7 months' maximum. The total duration of inclusion will be for 30 months. The follow-up of each patient will be determined according to their result obtained by the technique they were allocated to.

4. SELECTION OF THE PATIENTS

4.1 Inclusion Criteria

The following patients will be included in the study:

- 18 years old or over
- a singleton pregnancy
- a high risk for trisomy 21 estimated on the basis of the combined or sequential integrated screening (between 1/5 and 1/250)
- a gestational age between 11 and 18 weeks
- would accept a priori an invasive prenatal diagnosis,
- agree to be contacted again by questionnaire after the birth

- Accept a priori a karyotype if screen positive by conventional screening or following a positive NIPT result
- healthcare coverage in general social security system.
- accept and signs the informed consent.

4.2 Exclusion Criteria

The following patients will not be included in the protocol:

- those with a combined risk of $<1/250$ or $> 1/5$
- fetal nuchal translucency > 3 mm and/or a measure of PAPP-A and/or β hCG <0.3 MoM and/or > 5 MoM.
- fetal anomaly diagnosed at the 1st trimester ultrasound
- one of the parents being a carrier of a balanced chromosomal translocation (including chromosome 21)
- not desiring an invasive prenatal diagnosis of chromosomal abnormalities,
- participating in another trial,
- suspicion of a "vanishing twin "
- poor comprehension of the French language
- refusing to sign the informed consent.

5. PROGRESS OF THE STUDY

5.1 Patient recruitment:

The consulting patients or those referred to one of the participating centers with a risk estimated for trisomy 21 $> 1/250$ will be entered into a modified database and shared with the existing

BioNuQual base (<https://www.bionuqual.org/echo.php>) and will be offered to participate in the study.

The patients who fit the inclusion criteria and with no exclusion criteria, and having signed the informed consent will be randomized:

- The NIPT group will have a blood sample for sequencing of the cfDNA in the maternal blood. This blood sample will be performed instead of an invasive test which would have been proposed to them outside this research program (amniocentesis or CVS).
- The standard prenatal diagnosis group will have invasive fetal testing (amniocentesis or CVS) which will be performed and interpreted according to the local protocol of the hospital. The results will be given to the patient within the usual timeframe allocated and their future care will not be modified.

5.2 Technical modalities:

BioNuQual Database:

The Biologie Nuque Quality program called BioNuQual is a framework for the implementation of quality control for the prenatal screening of aneuploidies, which was introduced by the High Authority of Health ² and declared by order of June 23rd, 2009 thereby fixing the rules for best practice regarding screening and prenatal diagnosis of Trisomy 21 using maternal serum markers. The report by the High Authority of Health reflects on the importance of estimating the impact on the population the changes of strategy will bring for the screening of the trisomy 21. The order of June 23rd, 2009 ³ specified that within the framework of the follow-up of this screening, a procedure for data transmission was required to be established.

The objectives for this program include:

- to organize the exchange and the information sharing necessary for the quality control between the personnel of the prenatal screening.
- to estimate the quality of the practices of the sonographers and the laboratories regarding prenatal screening.
- to allow the feedback to the various practitioners and the public authorities.

BioNuQual adheres to 5 key principles defined by the Commission Nationale de l'Informatique et des Libertés (CNIL):

- i) The principle of purpose: the information concerning the patient is collected and handled only for a determined and justifiable use respecting the objectives of this program.
- ii) The principle of relevance of the data: the relevant and necessary information with regard to the objectives pursued by the treatment will be handled only.
- iii) The principle of a duration limited by preservation of the information: the information cannot be kept for an unlimited duration.
- iv) The principle of safety and data privacy.
- v) The principle of the respect for the patient's rights.

The information concerning the women at high risk is continuously recorded in the BioNuQual database which received a favorable decision from the CNIL (DE-2012-008).

The data collected in the database are listed in appendix 2.

Collection of the tests and the sample storage, the extraction of the DNA and the quantification of the total and fetal free DNA:

After inclusion in the study, every patient will have a sample of blood (20 ml) drawn into a tube that allows the circulating or cellular DNA to remain stable at room temperature for at least 7 days during transit ^{50,51}. This sample is taken in place of the invasive testing. Samples are shipped then immediately to the laboratory of cytogenetics at Hospital Necker-Enfants Malades where they undergo a first centrifugation at 1600xG for 10 minutes. The supernatant is then poured into a test tube to undergo the second centrifugation at 16000g for 10 minutes ⁵². These two stages of centrifugation are performed within 24-48 hours following the test, to maximize the preservation of the DNA ⁵³. The samples are then, if needed, kept in a freezer at -80°C ²⁸.

All the stages of extraction and quantification of the total and fetal DNA are performed in the department of cytogenetics at Hospital Necker- Enfants Malades. The extraction of DNA is performed with the extraction kit NucleoSpin ® Plasma XS kit (Macherey-Nagel, Düren, Germany) ⁵⁴ according to the recommendations of the manufacturer.

The quantification of the total and fetal DNA is done by performing in parallel, a real time quantitative PCR of a locus common to the mother and fetus for the quantification of the total DNA and for the SRY locus for the male fetus. To obtain the quantity of fetal DNA, we compare the quantity of DNA determined at the SRY locus in that of the common locus ⁵⁵. This technique allows the simultaneous determination of the fetal sex. When the fetus is female, the quantity of DNA cannot be determined.

- Parallel sequencing:

Parallel sequencing is a technique which allows sequencing of several thousands to several million fragments of a genome, simultaneously. This sequencing method will be performed on the sequencing platform Solexa/Illumina, installed at Hospital Necker-Enfants Malades.

The technique includes a stage of preparation of a DNA bank followed by the sequencing. At first, the extracted DNA is enriched in fetal DNA by a machine which separates DNA fragments according to their size (the circulating fetal DNA consists of smaller segments than those of the circulating maternal DNA). Then, universal adapters are used to amplify these segments and obtain millions of fragments covering the whole genome. Once this DNA bank is established, the sequencing stage can commence. The Solexa/Illumina system uses an approach to "sequencing by synthesis". All the sequenced fragments are then analyzed and compared with the chromosome reference sequences. Thus, this allows an analysis of the quantity of DNA resulting from the supernumerary chromosome, without the necessity of differentiating between the maternal and fetal DNA^{18,28}. The bank of fragments is performed by means of DNA Samples Prep Kit with some modifications with regard to the recommendations of the manufacturer. Indeed, the present DNA in the maternal plasma is already split up⁵⁶, therefore it is not necessary to perform a complementary stage of fragmentation. The sequencing is then carried out on the HiSeq device according to the recommendations of the manufacturer.

- Data analysis:

The parallel sequencing yields on average 10 million DNA fragments of approximately 36bp per patient. For each sample, sequences are compared with a genomic reference and those with more than one error are eliminated. We then apply a window of 50kb to every chromosome and determine the number of fragments in each window. The median of the number of fragments for every chromosome is retained.

The median of the autosomes is used to standardize the results of each chromosome, which compares the various samples to each other (the term " density of fragments " relates to the standardized value compared to the reference set = 30 chromosomally abnormal male fetuses).

In the case of the pregnancies where the fetus is carrier of a trisomy 21, an increase of the density of fragments on the chromosome 21 will be observed. This result is expressed in a Z-score compared with the reference set.

- Depiction and performance of result (see Figure 1):

The standard invasive group who undergo invasive testing (amniocentesis or CVS) and will be performed and interpreted according to local departmental protocol of each center. The results are returned to the patient within the normal expected timeframe and the subsequent care will not be modified. The result of the karyotype and the outcome of the pregnancy are collected and added to the database.

In the NIPT group, the result of the cell free DNA test will be available within a maximum timeframe of 3 calendar weeks. The results will be transmitted by telephone to the responsible doctor, confirmed by fax and by mail. The result will be also added to the BioNuQual database.

Three types of results will be possible:

A) The density of fragments of chromosome 21 expressed in Z-score is > 1.645 (5 % of the cases): the result of the NIPT will be considered as positive. A subsequent invasive test will be performed within eight days. The result of the karyotype and the outcome of the pregnancy are collected and recorded in the database. The chosen threshold for the quantification of the DNA of chromosome 21 is such that, the sensitivity for the detection of trisomy 21 will be > 99 %

and the specificity will be 95 % (false positive rate fixed to 5 %). The threshold of 1.645 was chosen to allow a decrease of the invasive testing rate by a factor of 10 - 20, meaning that an invasive test would not be performed in more than in 0.2-0.5 % of all the pregnant women. This threshold is, however, lower than that described in the literature, and used to limit the risk of false-negatives^{21,26,29,31,36,37,57,58}. As techniques progress, it seems that a sensitivity > 99 % can be maintained, and increasing this threshold decreases the false positive rate. Within the framework of the STIC SAFE 21, we can increase this Z-score threshold of 1.645 to 2.5 - 3 during the study, to guarantee a very high sensitivity while reducing the false positive rate, thus using a threshold similar to that reported in the literature.

B) The density of fragments of chromosome 21 is <1.645 (95 % of the cases): the result of the NIPT will be considered as negative. A standard ultrasound follow-up, in compliance with the recommendations will be performed⁵⁹. Indeed, after a negative result of NIPT, the individual risk of the woman is decreased by a factor of between 50 and infinity^{19,22,24,26,29-31,36,37} and the average residual risk of trisomy 21 will be of the order of 1/2000^{57,58}. The women can decide following a negative NIPT result, to undergo invasive testing, because she is not reassured by the result or a fetal anomaly is discovered at a later stage. This situation would only be expected in a low number of cases. The outcome of the pregnancy will be collected and added to the database.

C) The NIPT is not technically feasible or a result is not feasible within the time limits allowed (5 %)^{17-19,21,24,26,29-32,37}. An invasive test will be performed in under eight days. The result of the karyotype and the outcome of the pregnancy will be collected and recorded in the database.

Each patient will be given a self-questionnaire to complete on their attitude and preferences to NIPT. In particular, the advantages of NIPT over conventional karyotyping and vice versa. The data of the parallel sequencing will be kept on a secure server at Hospital Necker-Enfants Malades until the end of the study.

5.3 Follow-up of the patients - Modalities of surveillance

The duration of participation of every patient included in the trial is 7-months. This covers the period from eligibility to the end of pregnancy. In case of Fetal loss, a Fetal postmortem will be offered and performed with the patient's consent.

A clinical examination of the newborn child will be performed by a pediatrician in all cases and a karyotype with FISH from blood of cordon can be made in case of clinical suspicion of chromosomal abnormality.

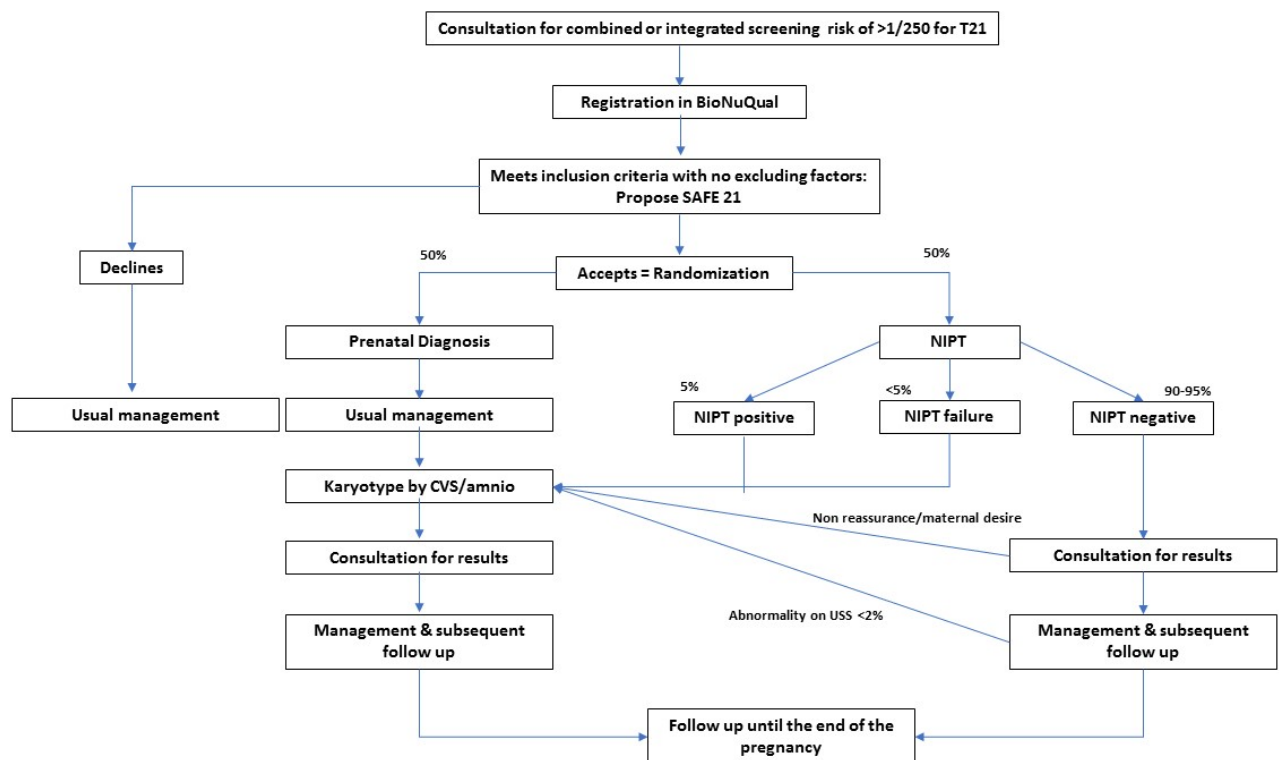


Figure 1. Study Flow Chart

6. CRITERIA OF EVALUATION:

Primary:

- Percentage of Fetal losses in each group

Secondary:

- Percentage of invasive taking in every group (amniocentesis or CVS)
- Diagnostic Performances of the NIPT, in particular the false positive and negative rates
- Percentage of result transmission of the NIPT within the agreed time frame and average delay of NIPT result delivery
- Percentage of failed tests or inconclusive results of the NIPT
- Percentage of other anomalies discovered if the invasive tests performed
- Percentage of invasive tests performed in spite of a reassuring NIPT (*ie* for a fetal anomaly, or for reassurance)
- Association between the maternal characteristics (weight, height, parity, maternal history, serum markers) and the result of the NIPT
- Costing of NIPT in clinical practice, and the monetary impact of its use with regard to conventional screening.
- Economic evaluation of the cost efficiency by expressing the result in € by avoided Fetal loss

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In practice, these centers are distributed nationally (5 centers in Ile-de-France, 22 provincial centers and an overall national grouping). All centers, screen patients using the first trimester combined performed according to the recommendations of the HAS (High Authority of Health).

The patients in the routine screening group will be managed according to the normal care path of the center. In the NIPT group, the result of the cell free DNA test will be available within a maximum timeframe of 3 calendar weeks. The results will be transmitted by telephone to the responsible doctor, confirmed by fax and by mail. The result will be also added to the BioNuQual database.

8. DATA MANAGEMENT AND STATISTICAL AND MEDICAL ECONOMIC ANALYSIS

8.1 Sample size

The main objective of this study is to obtain a significant decrease in the number of invasive tests performed, which is reflected by a significant reduction in the rate of miscarriages in the NIPT group. The required sample size to produce a decrease from 1.5 % to 0.5 %^{9,10} between the standard screening group and the NIPT group is 1250 patients (unilateral test with $\alpha = 0.05$ % and power = 80 %).

The participating centers have a total population of approximately 30,000 pregnant women a year presenting for first trimester screening, which in theory should lead to approximately 1,000-1,500 women per year at high risk. The participant acceptance rate for this study is close to 100 %, therefore the number of patients necessary should be over a period of 30 months.

8.2 Data Management

The data will be recorded and stored on the BioNuQual online electronic database. The data processing will be carried out by the Unity of Clinical Search (URC Necker) (Dr Laurence Bussières and Prof Treluyer).

8.3 Statistical Analysis concerning the main assessment criterion and the diagnostic performances

The analysis will be performed by Dr Caroline Elie (URC Necker) using the R software package (<http://cran.r-project.org/>). No interim analysis is planned. The statistical analysis will be performed at the end of the collection, and will include the capture and the consistency of the data.

A descriptive analysis of the characteristics of the patients included will be performed. The quantitative data are expressed in means \pm standard deviation or by medians with interquartile ranges, and number with percentages for the qualitative data.

Main assessment criterion

The rates of miscarriages will be compared by using the unilateral Mantel-Haenszel test with the risk set at 5 %. This analysis will be performed as intention to treat. The patients lost to follow up will be considered as having had a miscarriage, regardless of their allocated group of randomization.

A secondary analysis will be performed with the hypothesis of maximum bias (*ie* the patients lost to follow up will be considered as having had a miscarriage if they belong to the NIPT group and as having had no miscarriage if they belong to the routine screening group).

Secondary assessment criteria

For the secondary assessment criteria, all the used tests will be bilateral with a threshold of significance of 5 %. The comparisons of two means will be performed the student's t test or by the Wilcoxon Rank test if required . The comparisons of percentages will be calculated using the Chi-squared test or by Fisher's exact test. The evaluation of the diagnostic performances of the NIPT will be calculated as usual to determine the sensitivity, the specificity and the positive and negative predictive values. The reference test will be either the karyotype, or the phenotype for the birth. The 95 % confidence intervals will be calculated by Fisher's exact method (binomial law).

8.4 Economic analysis

This analysis will be performed by the ECO URC Ile-de-France (Prof. Durand-Zaleski)

8.4.1 Economic valuation methodology

The proposed analysis will be a cost-effective analysis as the quality of life linked to health is difficult to establish, except in the cases of the maternal anxiety and the maternal preferences. These will be estimated separately ^{48,49,60} (see section 8.5). The main hypothesis of the clinical trial is that the introduction of NIPT will 1) result in a significant reduction of the number of inferred miscarriages (main criterion of the clinical trial) 2) without reducing the number of trisomy 21 cases diagnosed.

The cost of performing an invasive test for women at high risk will be compared to the strategy that adds NIPT before the performance of an invasive test which would become necessity in the portion of the women with a risk $> 1/250$. The cost-effective criteria under evaluation are:

- The number of miscarriages (main criterion)
- The number of cases of trisomy 21 diagnosed

- The number of avoided invasive tests (amniocentesis or CVS)
- The reduction of maternal anxiety associated with the procedures and the preferences of the women (see section 8.5).

8.4.2 The choice of the approach

This reserved approach is the one of the system of care. It is coherent with the objectives of the call for tenders STIC. We will collect the resources allocated for screening and diagnosis, as well as the sequelae following screening up until delivery. The consultation data, the number of investigations performed and admissions to hospital in the participating centers of the study will be collected.

8.4.3 The choice of the population of analysis

The data will be collected prospectively for all women included in the study. Economic analyses for specific populations is not planned.

8.4.4 The choice of the interventions to compare

The reference test is the invasive procedure which follows a 1st trimester combined screening risk of $> 1/250$ between 11+0 and 13+6 weeks' gestation, recommended by the High Authority of Health since 2007. This reference test will be compared with a strategy of introducing NIPT prior to performing any invasive procedure.

8.4.5 The choice of the temporal horizon

The follow-up of the patients begins after the intervention up until childbirth. This duration was chosen to identify the outcomes of the screening and to validate the performances of the NIPT.

The longer-term consequences of termination of pregnancy or the birth of a child with trisomy 21 have been described elsewhere and are beyond the scope of this project⁶¹⁻⁶⁴.

8.4.6. The updating

The duration of follow-up will be for less than 1 year and it will not be necessary to update the costs and the results. If a model is used, the rate of updating will be 4 %.

8.4.7 The data used for economic evaluation

The consummate resources will be collected in the database for observation for all women included in the study.

8.4.8 The identification and the measure of the results

The main criterion is the reduction in the number of miscarriages, this result will be collected prospectively and analyzed for each arm in an intention to treat manner. The secondary assessment criteria and, in particular the number of cases of trisomy 21 diagnosed, will also be collected for the clinical trial.

8.4.9 Cost estimate

Only the direct costs will be taken into account in the reference analysis and integrated into the ratio cost-result. This will allow a comparison with previous French studies on the 1st trimester combined screening⁶⁵.

8.4.10 The identification, measure and valuation of the direct costs in the reference analysis

Data collection and valuation:

A. NIPT:

The new technique will be valued by micro-costing, with a direct observation of the laboratory including the staff, the equipment and the necessary consumables. This direct observation will be performed during a day in the laboratory of cytogenetics of the Necker hospital.

The resources considered for the collection by direct observation are:

- the number of staff present and their job role
- the specific consumables of the analysis, for the other consumables we previously used a fixed package (specific to each laboratory) specific to their used period.

The personnel costs will be estimated by the median salary of each member of staff. The consumables will be valued at the purchased price (with an analysis of sensitivity, taking into account the potential discount on price negotiated by different centers). The fixed costs and the overheads will be calculated by looking at the annual cost of the functioning of the laboratory for the duration of the analysis (assuming an 8-hour daily use of the laboratory, this figure can be adapted according to local conditions).

The average cost of each analysis will be estimated by valuing the average quantities of resources, by costs per unit costs available, at the end of the study, in order to supply the decision-makers the necessary information for prospective future costings.

The hospital admissions (invasive procedures or terminations of pregnancy) will be valued from the ENCC by taking into account the length of stay of the patients included in the study.

- i. The daily cost will be estimated from the data of the ENCC and the length of stay of the patients of the GHM in the national base. This will be initially done by excluding the daily costs of technical medical procedures, which will be then reinstated in the calculation of the total cost.
- ii. This daily cost will then be multiplied by the length of stay of the patients extracted from database

B. The cost of the reference technique

The costings of the examinations and the consultations necessary for performing an invasive test after a 1st trimester combined screening test will be estimated from the nationally quoted prices.

C. The cost of medical care during follow-up

The hospital admissions will be recorded in the database. The PMSI data (discharge summary) for the stay, and the cost of the stay will be the one of the ENCC. The cost analysis of the consummate resources will be performed in a identical way for both arms of treatment.

8.4.11 Modelling

We planned the basic analysis for the duration of the pregnancy, without modelling for the long-term consequences of the birth of a child affected by trisomy 21 or by a termination of pregnancy followed by the birth of another child. Such a model would be beyond the scope of the requirements of the DGOS and does not correspond with the recommendations of the HAS in it's 2007 report ². The HAS has moved away from the idea of modelling the societal cost

avoided by the termination of a fetus affected by trisomy 21, or the cost to society resulting from a child born with trisomy 21 that was not identified by screening.

8.4.12 Data analysis, linking the costs and results

The presentation of the data relating to costs and results will not be integrated. The costs will be compared using parametric and non-parametric tests. The main assessment criterion is whether there is a reduction in the number of miscarriages. We shall therefore estimate the possible additional cost per avoided miscarriage. The recent publications on NIPT underline that there is a reduction in the risk of invasive testing and miscarriage²⁹. The main assessment criterion of the study that has been chosen is consistent with the data in the literature, and the choice of the criterion for the economic evaluation follows supports this choice, as the clinical experts have validated that the main benefit of the NIPT would be to reduce the risk of invasive testing and miscarriage. There have been several economic evaluations published on the prevention of miscarriages for various indications⁶⁶⁻⁶⁸.

This choice of economic evaluation with the clinical trial does not prevent a secondary analysis being performed. This analysis will estimate the incremental ratio of cost per additionally diagnosed cases. The HAS have estimated ratios in cost per number of additionally diagnosed T21 cases to be between €965-€1,930. These values can serve as reference for the interpretation of the results.

The URC Eco will be responsible for the statistical analysis of the data used for the economic evaluation. The statistical analyses will be performed as intention to treat. The analysis will be based on the cost of each strategy and intervention, with regard to the patient. The resources and the costs will be described per screening group and presented accordingly. The category-

specific variables will be presented in terms of numbers and percentages. The quantitative variables will be described by their mean and standard deviation or their median matched by the extreme values or by their interquartile range according to the characteristics of their distribution (the cost data is likely to be a beta distribution). The consumption of resources and the resulting costs will be compared by student t tests, Kruskal-Wallis or ANOVA. The uncertainty of the ratios will be estimated by bootstrapping ⁷².

8.5 Analysis of the attitude of the pregnant women and the decisions

This analysis will be performed by Valérie Seror UMR912 (SE4S) INSERM, IRD, Aix-Marseille university.

8.5.1 Attitudes of the pregnant women and the analysis of the decisions

The possibility of non-invasive prenatal testing (NIPT) for the trisomy 21 in women at high risk following 1st trimester combined screening, would result in a reduced number of invasive tests performed for karyotyping (positive NIPT result is approximately 5 % of the 5 % of women at high risk). Compared with the current situation, this could considerably reduce the number of Fetal losses attributed to the invasive test (main objective of the STIC). However, the women benefiting from NIPT would be "deprived" of a complete and diagnostic fetal chromosome analysis. Consequently, the introduction of NIPT is not without some limitations.

At present, pregnant women at high risk based on 1st trimester combined screening have the possibility of:

- A diagnosis of a chromosomal abnormality
- But at the price of a risking their pregnancy (superimposed 1 % risk vs 0.5 % on average);

The introduction of NIPT gives pregnant women at high risk based on 1st trimester combined screening the possibility of having

A targeted test for trisomy 21 (almost certain exclusion a trisomy 21 in case of a negative result) but without analyzing the full chromosomal complement,

- No risk to their pregnancy,
- A low risk of technical failure of the procedure requiring an invasive test for a Fetal karyotype.

A fetal karyotype by invasive testing is therefore only proposed to women for whom:

- NIPT would show a strong suspicion of trisomy 21 (positive NIPT)
- Failure of the non invasive procedure to yield a result (not possible technically).

The introduction of NIPT has therefore modified the options available for pregnant women who are found to be at high risk of trisomy 21 following routine 1st trimester screening. While the encouraging results of NIPT studies begin to raise the question of implementation into routine practice, there are very few studies examining maternal attitudes towards this test. The studies published show a positive attitude towards the possible reduction of the risks associated with invasive testing⁷³. The balance between this risk of fetal loss with invasive testing and that of the NIPT needs to be considered and pregnant women appear to have a preference for waiting longer to have a result with NIPT on the condition of avoiding any risk to the pregnancy⁷⁴. However, the advantages of the introduction of NIPT must be balanced, because pregnant women frequently place a high value on the information given to them about their pregnancy⁷⁴. For these women, the introduction of NIPT raises the question of their preferences in terms of deciding between risk-taking with a diagnosis and no risk with a highly accurate screening test.

A study on the successive decisions of screening (ultrasound and serum markers) and invasive diagnosis shows that the pregnant women, for whom a Fetal loss following an invasive diagnosis is worse than giving birth to a child with trisomy 21, appear to be passive in their preference for which test to perform⁷⁵. Yet, no significant difference of appeal to the invasive diagnosis had been noted in these women, suggesting the possibility that they "voted with their feet". For these women, the possibility of a non-invasive advanced screening test with a high detection rate for trisomy 21 was an acceptable option⁷⁶.

As a result of the small number of studies on the subject of the maternal preferences, **our project suggests studying their attitudes towards the NIPT for trisomy 21 and it will consider their attitudes towards invasive prenatal diagnosis**. More precisely, the objective of this part of the study will be to identify and to analyze the preferences of pregnant women with respect to the risk-taking for the pregnancy. Highlighting these trade-offs is fundamental in the promotion of the autonomy of these women (couples) in the decision-making. In this respect, our study will allow the identification the elements that may or may not influence the decision taken with respect to further testing following a high risk result.

Our project aims at exploring, using a self-questionnaire, the decisions and the attitudes of the pregnant women:

- Those having NIPT
- Those having an invasive prenatal diagnosis, which due to the inclusion in the STIC, are aware of the availability of NIPT.

For our study, a questionnaire will be completed by the pregnant women during their recruitment visit and then a second after childbirth.

In the reading of the design of our study, it seems that attitudes towards the non-invasive procedure expressed by the pregnant women who refuse the invasive diagnosis, cannot be explored. One of the limitations of our project is the analysis of the individual trade-offs between the risk of Fetal loss and a comprehensive diagnosis of possible chromosomal abnormalities. As a consequence of the difficulty these pregnant women have, as well as, the difficulty of the data collection, we have decided not to analyze this topic.

8.5.2 Questionnaires

In addition to the sociodemographic variables of the investigated, the questionnaire will integrate the following aspects:

- Attitudes towards prenatal screening
- Understanding the result of the NIPT and the invasive diagnosis
- Attitudes towards NIPT and towards the invasive diagnosis

This questionnaire asks questions on the successive decisions of screening and diagnosis as well as on the understanding of the result of the NIPT and/or the invasive diagnosis, the link with the clinical trial is essential to confront the declarative with the objectives.

8.5.3 Methodological organization

On an operational level, the data collection will be overseen by a person of the STIC involved in the clinical trial itself, which will facilitate correspondence between clinical data and data from the questionnaire.

The printing of the questionnaires and their distribution to the centers, as well as, the follow-up of the investigation will be piloted from the platform of investigation of the Monitoring center of health from the Region of Provence-Alpes-Côte d'Azur. This platform has a proven track

record in carrying out this type of health research. Once the questionnaires are constructed, they will be handled by the platform and they will be responsible for the computer graphics and printing. The platform will ensure regular contact with the centers involved for the collection of data, and they will organize and verify the data capture.

8.5.4 Statistical organization

The results of the questions on the Likert scales will be re-transcribed in numerical value with possible grouping of methods.

The statistical analysis of the data will, on one hand, consist in identifying profiles of attitudes regarding the method of ascending hierarchical classification. This method does not predefine groups but rather, groups the individuals in classes suggested by the data. From the calculation of the distances between individuals (considering the methods taken by variables integrated into the analysis), an optimal number of classes is defined on the basis of a criterion of maximization of the distances collate and of minimization of the distances intra-classes (SPAD software). The method aims to build classes which are contrasted with each other, and considers the variables selected for the analysis. Once identified, these profiles of attitudes will be characterized by the sociodemographic variables and the decision-making behavior; The average scores of anxiety of the pregnant women with each profile will be compared (parametric and non-parametric tests according to the distribution of the scores). The data relative to the understanding of the result of the non-invasive procedure (NIPT) and the invasive diagnosis will be analyzed by logistic regressions to identify significant factors that impact (who's attitude profile) on their understanding.

9. MANAGEMENT OF ADVERSE EVENTS

Definitions:

Adverse Events

Any harmful or unintended event arising at a person during the study, whether or not it is linked with the study.

- Adverse Effect

Unwanted effect in a study that is not due to a product mentioned in the article L.5311-1 (medicine, biomaterials and medical devices, medical devices of in vitro diagnosis, produced unstable impulsive persons, organs, fabrics, cells and produced by human or animal origin, produced cellular in therapeutic purpose): any unwanted event while participating in the research.

- Serious Adverse Event/Effect

Effect or unwanted event resulting in one or more of the following:

- Death
- An illness that alters a prognosis for survival
- An incapacity or a severe or sustainable incapacity
- A hospital admission or an extension of a hospital admission
- An anomaly or a congenital malformation
- Other: any unwanted judged effect as serious by the healthcare professional, in particular the events requiring an intervention to avoid one of the consequences noted above

- New fact

Any new safety data able to lead to a revaluation of the report of profits and the risks of the search, or which could be sufficient to envisage modifications, in the conduct of the search.

Specifics of the research

Adverse events which can be serious but not requiring immediate notification to the sponsor.

Regarding the natural and normal evolution of the pathology:

- Serious and non-serious adverse events reported during the pregnancy.
- Scheduled hospital admission or admission for the treatment of a pathology known before the inclusion in the study.
- Any serious adverse effect susceptible to be bound to treatments prescribed within the framework of the care during the follow-up of the search
- Adverse effects linked to a blood test:
 - Located pain
 - Located bruise
 - Vasovagal response

Serious Adverse Events (SAE) linked to the study

No serious adverse events are expected within the framework of this study.

10. LOGISTIC, LEGAL AND GENERAL ASPECTS

10.1. Role of the sponsor

It is defined by the law 2004-806 of August 9th, 2004. In this research, the AP-HP will be the sponsor and the Regional Delegation in the Clinical Search which assures the statutory missions and will have a decision-making role.

10.2. Submission of the protocol for ethical approval

In agreement with the article L.1123-6 of the Code of the Health, the research protocol will be submitted to a consultative Committee of Protection of the People in Biomedical Research for

the Paris and the surrounding area, after agreement of the sponsor (with the insurance certificate and the receipt). The opinion of this committee will be outlined in the form sent to the competent authority by the sponsor prior to starting the study.

10.3. CNIL (NATIONAL COMMISSION FOR INFORMATION TECHNOLOGY AND CIVIL LIBERTIES) declaration

The law plans that the statement(declaration) must have been made before the actual beginning of the search(research).

The DRC (Direction of clinical research direction as sponsor will make a declaration to the CNIL (NATIONAL COMMISSION FOR INFORMATION TECHNOLOGY AND CIVIL LIBERTIES), in connection with the person in charge of the research database, that the research will be subjected to quality control by ARC (clinical research advisor) and complying with the CNIL procedures.

This project will not include identifiable genetics, epidemiology or the study of the behavior, which could contain critical confidential (ie patient identities of or Social Security Numbers). In this case, as well as for non-monitored research, the person in charge of the database will comply with the rules and regulations on research data processing set out by the CNIL.

10.4. Documentations of the research

Before starting the research, the investigator coordinator will supply a copy of his and the other investigator's personal curriculum vitae personal dated and signed and containing their medical registration number, to the sponsor of the study.

The version of the protocol accepted before submission with its appendices will be jointly signed by the investigator coordinator and the sponsor. Where necessary, the scientific advisor will be also be a signatory.

For every new version of the protocol, after the necessary amendments and/or requests of the authorities, a new number and the date will be attributed with the same meditative signatures.

Every investigator will make a commitment to respect the obligations of the law and to perform the research according to the B.P.C. and by respecting the terms of the declaration of Helsinki. A copy of the scientific commitment, signed and dated (document- DRRC) by each investigator of every participating center will be submitted to the sponsor.

10.5. Quality control and Quality assurance

The research will be supervised according to the standard operating procedure of the sponsor, AP-HP.

The progress of the research and the care of the subjects in the participating centers, will conform with the declaration of Helsinki and Good Clinical Practice.

Procedures of monitoring

The sponsor will visit the participating centers to ensure adherence to the protocol and patient follow-up.

Prior to recruitment, each center will be opened following a site initiation to train local staff and introduce the protocol. During the following visits, the sponsor will examine the electronic case record form to ensure the quality and validity of the data. The main investigator and their team for each center agree to welcome representatives of the sponsor appointed by the AP-HP at regular intervals.

During these planned visits and in agreement with Good Clinical Practice, the following elements will be revised:

- Adherence to the research protocol and its defined procedures

- Examination of the source documents and examination of the data in the electronic case record form, quality assurance of the data collected in the electronic case record form: accuracy, missing data, coherence of the data, according to rules set out by the procedures of the DRRC.

10.6. Amendments to the research protocol

The DRRC must be informed of any modification of the protocol by the investigator coordinator. The modifications must be substantially qualified. Any amendment to the research protocol, must be communicated to the CPP if it entails substantial modifications. This includes situations where the planned modifications may, somehow or other, modify guarantees brought to the people who lend themselves to the biomedical research (modification of a criterion of inclusion, extension to the duration of inclusion, participation of new investigators ...).

10.7. Extension of the research

Any extension of the research (extensive modification of the protocol or the inclusion criteria, extension of treatments and or therapeutic acts not planned initially in the protocol) must be considered as new research.

10.8. Responsibility

The Assistance Publique-Hôpitaux de Paris is the sponsor of this research. In agreement with the law on the biomedical researches, an insurance policy with GERLING has been taken out

for the duration of the study, guaranteeing its civil liability as well as that for each participant (doctors or staff involved) in the research (law n°2004-806, Art L.1121-10 of the CSP).

The Assistance Publique-Hôpitaux de Paris reserves the right to interrupt the study at any time for medical or administrative reasons; in this eventuality, a notification will be supplied to the investigator.

10.9. Final report of the research

The final report of the research will be written in collaboration with the coordinator and the biostatistician for the study. This report will be submitted to each of the investigators for their opinion. When a consensus is reached, the final version must be endorsed by the signature of each of the investigators and sent to the sponsor as soon as possible after the effective end of the research. A report drafted according to the reference plan of the competent authority must be submitted to the competent authority as well as to the committee within one year, after the study has finished. This deadline is reduced to 90 days in case of a premature end to the research.

10.10. Publications and Intellectual Property of the data

The AP-HP is an owner of the data and no use or transmission to a third can be made without its preliminary agreement.

The authors of the publications will be the researchers heavily involved in the design, recruitment of patients, follow up of included patients, collection of data, and analysis of the results.

The Assistance Publique - Hôpitaux de Paris must be mentioned as being the sponsor of the biomedical research and as financial support if necessary. The terms " L'Assistance Publique - Hôpitaux de Paris " must appear in the address of the authors.

Appendices:

- 1- Letter from Pr J Weissenbach to Pr M Vekemans, detailing the technology transfer of Genoscope in the Laboratory of Cytogenetics of the hospital Necker.
- 2- Technical specifications of BioNuQual

The BioNuQual programme is saved on a secure anonymous health database with information relating to the prenatal screening of aneuploidies. The company HORIZON assures the maintenance of the study using logs, external monitoring of data and the verification of MySQL tables. The maintenance of the operating system and application software is made in a purpose of evolutionary reassurance and reliability by its compatibility with the hardware. A hotline is available for the supervisor.

The following data will be recorded:

1. Number of the approved laboratory
2. Number of patients attributed to the laboratory
3. Date of birth of the patient
4. Date of the 1st trimester ultrasound scan
5. Sonographer certification number
6. Value of the Crown Rump Length (CRL)
7. Value of the Nuchal Translucency (NT)
8. Date of the blood sampling
9. Value of PAPP-A and its MoM in the 1st trimester
10. Value of the BhCG and its MoM in the 1st trimester
11. Value of the NT and its MoM in the 1st trimester

12. Value of the BhCG and its MoM in the 2nd trimester
13. Value of the AFP its MoM in the 2nd trimester
14. Value of the E3 its MoM in the 2nd trimester
15. Result of the calculation of the combined risk or the integrated sequential risk or the risk calculated from the only serum markers in the 2nd trimester

The data are stored on a secure server. Firewall filters the access to all the departments. The HTTP and HTTPS are open to all. This is in compliance with the recommendations of the CNIL. The server is a Linux server completely dedicated to the site BioNuQual according to the recommendations of the CNIL. The CNIL decision DE-2012-008 authorized the implementation of data processing of personal health, in order to evaluate the practices of the prenatal screening of aneuploidies (Authorization request N 1529842). BioNuQual respects 5 key principles defined by the Commission nationale de l'informatique et des libertés.

An interface tool, accessible by any web browser, allows access to information according to what is required. MySQL is the program dedicated to the management of the data (storage and request of results). It can store large quantities of information, as well as, facilitating complex requests for reports or statistics on this data. PHP and MySQL allows to deploy solutions centralized on the server. PHP offers an ergonomic manipulation of the data with graphic possibilities. MySQL can: store any type of data, research multicriteria and also assure the function of reporting on a large quantity of data. PHP provides the graphic display of the figures, the construction of tables for the relevant periods while MySQL takes care with supplying the results to the PHP.

The data are saved on a daily and weekly basis. These automatic weekly data saves are stored for a minimum of 10 months.

The access to the data is made through the internet via an encrypted SSL. The passwords contain a minimum of 12 digits minimum. The consultation of results can be directly made by the user. Every consultant is limited to the data that the BioNuQual workgroup has predefined.

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