St George's University Hospitals NHS
NHS Foundation Trust

SW Thames Regional Genetics Laboratory

The SAFE Test Laboratory
User's Manual

Document code	GEN-SAFE-LAB-13
Version number	.01
Issue date	25/07/17
Review interval	See Q-Pulse for review date
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Distribution	Electronic copy: Q-Pulse. MyNIPT Portal, theSAFEtest.co.uk Paper copies: 01.243B

Document Revision History

Version	Date	Summary of Change	Revised by

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1 Introduction

The St Georges Antenatal Fetal Evaluation (SAFE) test Laboratory is located in the St George's University Hospitals NHS FT and is part of the South West Thames Regional Genetics Laboratory.

The SAFE test laboratory runs the CE-IVD IONA® screening test developed by Premaitha Health. It is an *in vitro* nucleic acid screening test that measures the likelihood that a woman is carrying a fetus with Trisomy 21, 18 or 13. The test is intended to be used by a clinician, in combination with other risk factors, to estimate the risk of an affected pregnancy.

The SAFE test is not intended to be used as a diagnostic test to confirm the presence of fetal Chromosome 21, 18 or 13 Trisomy.

The SAFE Test screens maternal plasma samples for fetal aneuploidies, using automated next generation sequencing. The use of plasma as sample matrix allows the SAFE Test to reduce the need for invasive prenatal testing, whilst providing women with accurate, reliable information on which to base their decisions.

This laboratory does not perform tests for any other disease. Clinicians can request SAFE test with the patient's consent. The following details are provided for the users to aid their referral process.

2 Contact information

2.1 Postal address

All samples and correspondence should be addressed to:

Genetics - the SAFE test Laboratory
Jenner Wing – Basement – Room 01.242
St George's University Hospitals NHS FT
Blackshaw Road
London
SW17 0QT

2.2 Other contact information

Telephone: +44 (0) 20 8725 5864 /+44 (0) 20 8725 5874
Fax: +44 (0) 20 8725 2138
Email: thesafetest@nhs.net/ theSAFEtest-report@nhs.net
Website: www.theSAFEtest.co.uk

3 Key People

Name	Description	Contact details
John Short	Consultant Clinical Scientist	John.Short@stgeorges.nhs.uk
Basky Thilaganathan	Professor & Consultant Obstetrician Lead	basky@pobox.com
Joanne Hargrave	SAFE Test Midwife	Joanne.Hargrave@stgeorges.nhs.uk 07876710540

4 Laboratory hours of operation

The laboratory is open Monday – Friday, 9.00 am to 5.00 pm excluding bank holidays. Samples will not be received outside of these times.

5 Clinical services offered by the SAFE test laboratory

5.1 Principals of the testing procedure

The testing is based on IONA® test proprietary to Premathia Ltd. No other tests are carried out.

The analysis is performed on cell-free placental DNA from maternal blood samples. Samples are taken in Streck tubes and transported to the laboratory for processing. The plasma is separated from the other blood components and maternal and fetal DNA is extracted. The DNA is then barcoded (for identification), amplified and processed enabling Next Generation Sequencing (NGS) on the ION Proton. The automated analysis step takes the NGS data and directly measures the change in the proportion of chromosome 21, 18 and 13. This is then reported as a likelihood risk.

5.2 Tests offered

The laboratory carries out testing on peripheral blood of pregnant women to reveal the carriage of a fetus with three genomic abnormalities: Down's syndrome (T21), Patau's syndrome (T13), and Edward's syndrome (T18). The SAFE Test is suitable for women who are at least 10 weeks pregnant with either a singleton or twin pregnancy. The SAFE test is not suitable for multiple pregnancies (greater than twins), or if the mother has cancer or a chromosomal or genetic abnormality (including Down's syndrome).The accuracy of the SAFE Test may be affected if the mother has recently undergone a blood transfusion in the last 3 months or had transplant surgery, immunotherapy or stem cell therapy prior to the blood sample taken. Results may be confounded in the case of placental mosaicism, partial trisomy or translocations, interuterine fetal demise/disappearing twin or if the mother has cancer.

The SAFE test has a detection rate of over 99% for Down's syndrome, 99% for Edward's Syndrome and 99% for Patau Syndrome.

In some instances, a diagnostic test such as chorionic villus sampling **(**CVS**)** or amniocentesis should be considered. It is recommended that all screening options, risk factors and results are discussed with a healthcare provider. In the event of a high-risk result (indicating that there is an increased chance that a baby will be born with Down's, Edwards' or Patau's syndrome) it is important to verify and confirm the high-risk result by CVS or amniocentesis.

The SAFE test offers optional testing for private referrals to determine the baby's sex with a sensitivity of over 99%. However, in about 3% of cases, it is not possible to issue a fetal sex report because of natural sex chromosome variations in the placenta. The SAFE test is not suitable for detection of sex chromosome abnormalities and sex determination is not currently available for multiple births (two or more babies) except for identical twin pregnancies.

5.3 Specimen requirements

Specimen	Sample Collection	Transportation
STRECK (Cell-free DNA BCT CE)	Collect **one tube** of 10ml STRECK blood per patient; ensure tube is **labelled** with 3 identifiers. Smaller volumes are accepted, but this may adversely affect the result of tests performed.	Transport at room temperature (6-37deg), stable up to 14 days at room temperature.

6 Requesting tests

All requests should be made using the SAFE test referral form; informed consent for testing should be filled in for each patient. Printable copies of this form are available from the website, the MyNIPT portal or directly from the SAFE Test Lab. Please complete all parts of the form.

Prior to signing informed consent for genetic testing, details should be explained to the patient by informed medical personnel (e.g. midwife, sonographer). The patient(s) should also be informed of the turnaround times of the screening test.

The following guidelines should be used for completing any request form.

	Essential	Desirable
On Sample Container	• Patients full name – spelled correctly • Date of Birth • Hospital or NHS number • Date and Time	
On Referral form	• Patients full name – spelled correctly • Date of Birth • Age • Current gestation age • If IVF pregnancy – age of mother/donor at egg harvest • Chorionicity details • Referring hospital details and name of referrer • Blood draw date • Patient consent • Clinician signature	• Patients Address and contact number • Maternal weight and height • Ethnic Group • Prior screening test results • Relevant medical history

Please confirm the patient identity before taking the sample

7 Sampling handling information

Prepare the sample as detailed below. **All samples should be labelled with at least 3 identifiers (First name, surname and date of birth and/or hospital no) or they may be rejected.**

- Complete a 'Screening Request and Patient Consent' form for each sample collected.
- Draw 10mL blood sample from pregnant woman into a 'Streck' blood tube (preferably).
- Mix immediately by inverting several times (x10).
- Record onto blood sample tube:
    - Patient ID, Patient date of birth, Blood draw date, If not posted immediately store at room temperature (6-37ºC) (**do not refrigerate**)
- Place the blood sample tube into specimen mailing tube containing absorbent material, place into the biohazard bag.
- Place biohazard bag and 'Screening Request and Patient Consent' forms into the padded envelope (for clinics outside of St Georges Hospital) labelled with the SAFE

test address and a UN3373 Biological substance category B label (information provided below).

Sample collection component check list:

- Blood tube - Cell-Free DNA BCT® ("Streck").
- Specimen mailing tube with absorbent material (outer tube).
- Biohazard bag.
- Padded envelope for clinics outside the St Georges Hospital.
- "Screening Request and Patient Consent" form.
- Brochures Information for NHS and Private Patients.

Additional sampling handling information is available in the document below and is also available on the MyNIPT Portal.

Sample Handling
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8 Transportation of SAFE test samples

Ensure that a SAFE Test Lab **Request Form** is completed for each patient, providing patient, referral, sample and clinical details along with informed consent for genetic testing.
Place the sample(s) and associated forms in outer tube and a clear sample transport bag. Place this bag in a padded envelope or a transport container, UN3373 compliant as required. See below for UN3373 instructions. Send the sample to:

Genetics - the SAFE test Laboratory
Jenner Wing – Basement – Room 01.242
St George's University Hospitals NHS FT
Blackshaw Road
London
SW17 0QT

- **NOTE**: If a courier is used for delivery please specify delivery directly to the SAFE Test Lab or to the CENTRAL PATHOLOGY RECEPTION at St Georges Hospital.
- Register the sample on the **My NIPT™ portal.**

UN3373 - Summary of Packing Instruction 650 (PI650)

SAFE test sample transport should comply with UN3373, details below;

The packaging needs to be strong enough to withstand shocks normally encountered during transport, transfer and mechanical handling. It should prevent loss of contents due to vibration, or change in temperature, humidity or pressure. There are 3 major components:

- **Primary leak-proof container** (i.e. sample/blood tube) – each sample must be individually wrapped to prevent contact if multiple samples are sent in one package.
- Absorbent material (tissue paper or cotton wool) – enough to absorb the entire contents of the primary containers.
- **Secondary packaging** (plastic tube and/or a sealed specimen bag) – leak proof to protect the outer packaging. Any paperwork should be outside this container.
- Cushioning material – to secure the secondary packaging within the outer packaging.
- **Outer packaging** – This must be rigid with at least one surface having a minimum dimension of 100x100 mm. They must pass a "drop test" of 1.2 meters when

containing primary and secondary packaging. They must be labelled with the following symbol and text:

BIOLOGICAL SUBSTANCE, CATEGORY B

The sides of the diamond must be at least 50mm, the width of the line at least 2mm and the text within and adjacent to the diamond at least 6mm high. For full instructions go to www.hse.gov.uk/biosafety/biologagents.pdf.

If samples are being transported from hot climates please contact the SAFE test laboratory for advice on appropriate transport methods to ensure the samples arrive in an adequate state to process.

9 SAFE test laboratory criteria for accepting and rejecting samples

The receipt of unsuitable samples may result in sample rejection, re-sampling request, poor sample quality, delays in tests/results and/or possible delivery of wrong diagnosis. In case of doubt please contact the SAFE Lab. Information on lacking data must be noted on the referral form and the referring clinician contacted. Samples will be checked against a set of criteria [GEN-SAFE-LAB-01].

Criteria for Rejecting Bloods	**Samples for SAFE test analysis**
Less than 3 identifiers	Samples inadequately labelled <3 identifiers, note on referral form and inform senior member of staff, these will likely be rejected.
Broken tubes	All blood samples in broken tubes should be rejected and should be handled as per SOP GEN-ALL-H&S-02. Note on referral form and query with senior staff member if sample discard required.
Compromised samples	Samples taken in the incorrect tube or transported incorrectly (ie received not at room temperature – on ice) or received compromised (cracked tube).
Clotted samples	Unsuitable for SAFE test analysis and a sample in an appropriate tube should be requested. Inform senior member of staff and they will inform booking in staff if sample discard required.
Old samples	DNA can be extracted from the plasma from <14 day Streck samples. If the sample is outside of this criteria inform a senior member of staff and a repeat sample will be requested, they will inform booking in staff if sample discard required.
Gestational Age	<10 weeks gestation.
Heparin	If a patient is on a dose of >7500iu of Fragmin (Dalteparin) this could lead to an invalid result.
Multiple pregnancies	Test is not suitable for multiple pregnancies (greater than twins).

10 Factors known to significantly affect the performance of the examination

The following key factors may affect the performance of our tests or interpretation of the results achieved:

Specimen factors:

- Low blood volume – inadequate quantity of DNA.
- Clotted blood – low quality/quantity of DNA.
- Wrong type of specimen container.
- Delayed sample transport to lab.

Clinical factors:

- Incorrect/inaccurate clinical information/diagnosis, family history.
- Presence of low level mosaic mutations in founder individuals.
- The SAFE test is not suitable for multiple pregnancies (greater than twins).
- If the mother has cancer or a chromosomal or genetic abnormality (including Down's syndrome).
- Unsuitable for mothers who have undergone a blood transfusion in the last 3 months, or had transplant surgery, immunotherapy or stem cell therapy.
- Less than 10 weeks of pregnancy.

11 Results and reporting

- Only qualified staff can authorise test results.
- Test results, consisting of a report per patient, are uploaded on to the **My NIPT™ portal** as soon as the test is completed and reports generated.
  My NIPT™ portal - https://mynipt.com
  - If you are unable to download reports from the portal please contact the safe test laboratory or the portal hosts; support@bluehub.co.uk.
  - For advice regarding use of the portal please contact the safe test laboratory, +44 (0) 161 232 5600 or email techsupport@premaitha.com
- Urgent reports can be faxed or emailed from theSAFEtest@nhs.net to a secure location upon request.
- No telephone reports are issued because of the risk of error in verbal transmission.
- Amended reports are issued in cases where an error has been discovered in an already released report or when additional information is obtained/ additional testing is completed where new information needs to be added to an already released report.
- Amended reports are marked as "revision 1 or 2 … etc." compared to the original statement "revision 0" and are accompanied by a cover letter stating the amendment to the original report.

Low Risk: means that it is very unlikely that your pregnancy is affected by trisomy 21, 18 or 13, and therefore very unlikely that your baby has Down's, Edwards' or Patau's syndrome.

High Risk: means that your pregnancy is at increased risk for trisomy 21, 18 or 13 and that the result should be confirmed by an invasive diagnostic test, such as amniocentesis or CVS to give a definitive diagnosis.

Failed Test: in a very small number of cases tests may need repeating due to insufficient placental DNA in the mother's blood. Under these rare occasions another blood sample will be required.

12 Expected turn-around times

Please note the following comments:

- Turn-around times are from receipt in the laboratory until a report is available electronically.
- Most samples are processed on the same day that they are received.
- The minimum processing time for a sample in the laboratory is 72 hours.
- An additional day of testing is required for requests that include fetal sex determination.

13 Reports available on request

- External quality assurance.
- Turn-around time compliance.
- Accreditation information.

14 The laboratory's policy on protection of personal information

The SAFE test laboratory complies with the guidelines and policies of St Georges Hospitals NHS Foundation Trust. Please see the St Georges University Hospital FT website for details of how the trust complies with the Data Protection Act etc.

15 The laboratory's complaints procedure

If there is an issue that needs to be raised with the department please contact the laboratory on 02087255864 and ask to speak to the laboratory manager. Alternatively email theSAFEtest@nhs.net. All complaints will be forwarded to the Head of the Laboratory and Quality Manager and wherever possible they will be dealt with straightaway.

In cases of a formal complaint through the Trust, the Trust Complaints and Concerns policy and procedure (Org 2.6) will be followed. Please also see trust website for the complaints process.

16 Clinical advice and interpretation

The SAFE Test Lab is contactable for any clinical advice during operational hours. Information leaflets are also available in the lab and in clinics offering the test.