GUIDELINES FOR THE REFERRAL OF SICKLE CELL AND THALASSAEMIA PRENATAL DIAGNOSIS SAMPLES TO MOLECULAR HAEMOGLOBINOPATHY LABORATORIES

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Version 2.4

CONTENTS

INTRODUCTION

The NHS Sickle cell and thalaasaemia screening (SCT) programme was set up in 2001 with the aim to develop a linked high quality screening programme. During this time the screening programme has worked closely with the three molecular haemoglobinopathy laboratories to offer a high quality prenatal diagnostic service to at risk couples identified through screening. The programme has worked with the laboratories and regional teams to develop this set of guidelines for the referral and follow up of sickle cell and thalassaemia prenatal diagnosis samples to molecular haemoglobinopathy laboratories. The guidelines cover three areas:

- **Part One: Referral of samples**
- **Part Two: Collection of Outcome data**
- **Part Three: Laboratory contact details and referral forms**

PART ONE: REFERRAL OF SAMPLES

CONFIRMATION OF RISK

Fetal sampling should only be carried out if the risk to the pregnancy is confirmed. In some instances this is straightforward e.g. risk of sickle cell disease when both parents are carriers of sickle cell, as sickle cell status can be easily confirmed by most routine diagnostic/screening haematology laboratories. Molecular confirmation of parental carrier status for alpha and beta thalassaemia is highly recommended prior to fetal sampling to identify the mutations involved.

If there is any doubt about the parental genotypes or whether further testing is required prior to fetal sampling, please contact the molecular haemoglobinopathy laboratory for advice.

NOTIFYING THE MOLECULAR HAEMOGLOBINOPATHY LABORATORY

The molecular haemoglobinopathy laboratory must be contacted in advance to make arrangements for the referral of a prenatal diagnosis sample. This ensures that the laboratory knows to expect the sample and can follow-up if the samples do not arrive at the appropriate time. See Part three of these guidelines for contact details of the laboratories.

REQUEST FORM

A fully completed prenatal diagnosis request form must accompany the fetal/ parental samples. Additional information such as antenatal screening results or genetic results from other laboratories should be included, particularly in the absence of a paternal sample. See Part three of these guidelines for copies of request forms.

PARENTAL BLOODS

Ideally new bloods from **both** parents in this pregnancy (2 x 5 ml EDTA) should be sent with each prenatal diagnosis sample. These can either be sent to the molecular haemoglobinopathy laboratory ahead of the fetal sampling or in cases where the parental mutations are known (e.g. carriers of sickle cell) can be sent with the fetal sample.

Prenatal diagnosis is not possible without a maternal blood sample as this is required to confirm the maternal genotype and to exclude significant maternal DNA contamination of the fetal sample.

If the paternal genotype is known (i.e. the father has been previously tested in another laboratory) but he is currently unavailable for blood sampling, a copy of the father's laboratory results should be sent to the prenatal diagnosis laboratory so they can assess the fetal risk.

If the paternal genotype is unknown and he is unavailable for testing, prenatal diagnosis can still be carried out but the conditions that can and cannot be excluded will be complex and depend on factors such as maternal genotype, family origins etc. The potential risk to the fetus in such cases should be discussed carefully with the molecular laboratory **prior** to fetal sampling being undertaken. Results will usually be presented on a risk basis and extended testing may be required which could delay the turn around time. Mothers/ couples should be informed of possible timescales and delays by the responsible clinician.

New parental bloods must be taken in every prenatal diagnosis even if the couple have had prenatal diagnosis previously. This overcomes issues with insufficient samples being stored and pregnancies with different partners. If the father is unavailable for blood sampling and the referrer requires the haemoglobinopathy lab to use a stored paternal DNA sample then the referrer must contact the laboratory directly and confirm the details of the father for the current pregnancy in writing (preferably on the request form).

FETAL SAMPLES

CVS, amniotic fluid or fetal blood can be used to extract fetal DNA to carry out the prenatal diagnosis. In most instances sufficient DNA can be obtained from un-cultured fetal material to obtain a diagnosis. This allows results to be turned around within a few days (see below). However in some cases the obstetrician is only able to obtain a very small fetal sample, which may mean the sample will need to be cultured in order to obtain enough DNA to carry out the diagnosis. Parent's and health professionals must be aware that this eventuality will result in a much longer turn-around time for results as cultures normally require 10-14 days to grow.

CHORIONIC VILLUS BIOPSY SAMPLE (CVS)

The CVS must be cleaned by microscopic dissection to remove any contaminating maternal tissue before being used for fetal diagnosis. The referrer should arrange with their cytogenetics laboratory for the sample to be cleaned and forwarded by guaranteed post or courier to the molecular laboratory with the appropriate documentation (i.e. prenatal diagnosis request form). It is recommended that the cytogenetics lab sets up CVS back-up cultures in-case there is insufficient DNA in the un-cultured material to carry out the diagnosis. The molecular laboratory will contact the cytogenetic laboratory if and when the backup cultures are required.

AMNIOTIC FLUID SAMPLE

Obstetric departments should aim to take approximately 20 mls of amniotic fluid which can be split between the cytogenetic and molecular laboratories. 10mls should be forwarded directly to the molecular laboratory for testing and the remaining 10mls sent to a local cytogenetics laboratory for back-up cultures/ karyotyping.

(Alternatively, all the fetal sample can be sent to the cytogenetic laboratory for them to divide and send the sample to the molecular laboratory). The molecular laboratory will contact the cytogenetic laboratory if the backup cultures are required. If it is not possible for the obstetrician to obtain 20mls of amniotic fluid the molecular laboratory must be notified.

FETAL BLOOD

On very rare occasions fetal blood sampling may be performed and a fetal blood sample sent in EDTA for analysis.

TURN AROUND TIMES

If the parental genotypes are known the target turnaround for prenatal diagnosis is 3 working days upon receipt of the fetal sample in the molecular laboratory. If the parental mutations are not known prior to fetal biopsy then the turnaround time is likely to be longer.

Please note: this target time does not include sample preparation time, in cytogenetics laboratories or sample transit times which may vary between centres Also, if a fetal sample is small and cultured cells are required to complete the diagnosis then the turn-around time for results will be much longer as cultures normally require 10-14 days to grow (see section on fetal samples).

REPORTS

When the fetal diagnostic report is ready the main contact for results will be alerted and the report can then be sent via secure FAX or encrypted email. Hard copies of reports will then be sent out by post. It is important for referrers to be aware that all prenatal diagnosis results assume the stated family relationships to be true. The molecular lab assesses fetal risk and defines the appropriate genetic testing approach from the information supplied. Inaccuracies in stated relationships such as non-paternity can lead to a misdiagnosis.

PART TWO: OUTCOME DATA COLLECTION GUIDELINES

As part of national quality assurance, all molecular laboratories report anonymised pregnancy outcome data on women that have undergone prenatal diagnosis for sickle cell and thalassaemia to the NHS Sickle cell and thalassaemia screening programme. This allows the prenatal and newborn screening results to be compared, as well as providing information on the choices made by families. The check with the newborn screening laboratory is done by the molecular PND laboratory but is only possible when information about the pregnancy outcome is provided by the maternity unit where the woman is booked.

PROCESS TO ASSESS OUTCOMES

A form to support collection of outcome data has been developed (see appendix One). This is a two part form that is sent by the molecular laboratory to the requesting clinician with the PND result. On receipt the requesting clinician should ensure:

- Part 1 is completed by screening co-ordinator or specialist nurse and returned to the molecular haemoglobinopathy laboratory within 1 month of the PND being reported. Information on whether the pregnancy is continuing, has miscarried or been terminated is required.
- Part 2 is retained by screening co-ordinator or specialist nurse until delivery, if the pregnancy is on-going.
- Part 2 B is completed by the Screening co-ordinator or specialist nurse within one month of delivery and returned to molecular haemoglobinopathy laboratory. Information on the baby's date of birth, NHS number and place of birth is required. This allows the molecular haemoglobinopathy laboratory to contact the appropriate newborn screening laboratory to obtain the newborn screen result

This process is essential to compare and link the prenatal result with the newborn screening result. Maternity units and referral/tertiary centres should work together with the molecular laboratories to ensure that completed outcome data is returned to the molecular laboratory in a timely manner so that quality assessments across the pathway can be made.

PART THREE: LABORATORY CONTACT DETAILS

John Radcliffe Hospital Oxford

Contact Dr Shirley Henderson, Principal Clinical Scientist/Deputy Director

email: hbopathy.screening@nhs.net or molhaem@ouh.nhs.uk

Tel 01865 572769

Fax 01865 572775

Address: National Haemoglobinopathy Reference Laboratory

Molecular Haematology

Level 4

John Radcliffe Hospital

Oxford

OX3 9DU

Website with request form:
http://www.oxfordradcliffe.nhs.uk/forpatients/departments/labs/haematology/molhaem/haemoglobinopathies.aspx
An example request form is given on page 15 of this document

King's College Hospital

Contact Dr Barnaby Clark, Principal Clinical Scientist
email: barnaby.clark@nhs.net
Tel 020 3299 4337

Tel lab 020 3299 2265

Fax 020 3299 1035

Address: Red Cell Centre

1st Floor Bessemer Wing

King's College Hospital

Denmark Hill

London SE5 9RS

Website: http://kingspath.co.uk/

Request form: available at http://kingspath.co.uk/tests/haematology/58/ and on page 12 of this document

University College London Hospital

Contact Dr Mary Petrou, Director Haemoglobinopathy Genetics Centre

email: mary.petrou@uclh.nhs.uk Haem.Gen@uclh.nhs.uk

Tel 020 3447 9458

Fax 020 3447 9864

Address: 86-96 Chenies Mews
London WC1 E6HX

Paper copy of request form available on page 13 of this document

NHS
Antenatal and Newborn Screening Programmes

Dear requesting clinician,

As part of Quality Assurance, all PND laboratories are collecting pregnancy outcome data on women that have undergone prenatal diagnosis for sickle cell and Thalassaemia from 1 July 2008. The main aim is to link prenatal and newborn results. The linkage will be done by the PND lab, but the PND labs will require notification of the pregnancy outcome from the maternity unit where the woman is booked. *To achieve this, can you send this outcome form to the screening co-ordinator or specialist nurse at the maternity unit where the woman was booked.*

For more information on this work please contact the PND laboratory or the Sickle Cell and Thalassaemia Screening Programme Centre (020 7848 6634). Thank you very much for your help with this important work.

PND OUTCOME FORM (Part 1) - Short Term Pregnancy Outcome

Part A - please forward to requesting unit		**Outcome Form Unique number:**		
Maternal Surname	**First name**	**DoB**	**NHS Number**	**EDD**
Maternal Address		**GP Name and Address**		**PND reference** T

..... cut here .....

PND OUTCOME FORM (Part 1) - Short Term Pregnancy Outcome

Part B To be completed by the screening co-ordinator or specialist nurse and returned to PND lab within 1 month of receiving PND result	**Outcome Form Unique number:**		
Maternity Unit address	Date of referral	Referrers name	PND result
Please return to *PND lab address:*	**Please return by** *(one month from date of PND result)*		

Please tick outcome:

CONTINUING PREGNANCY [ ]
MISCARRIAGE [ ]*
TERMINATION OF PREGNANCY [ ]*

**If there is a miscarriage or termination of pregnancy, do not complete Part Two of the outcome form*

Completed by (please print) NAME TELEPHONE	**Date** Part 1 B completed on

Please complete parts in blue, retain the named portion of this form (part 1 A), and return Part 1B - Short term Pregnancy Outcome form, with it's unique identifying number to the PND laboratory

PND OUTCOME FORM (Part 2) - Final Outcome

Part A - please forward to requesting unit		**Outcome Form Unique number:** OXT		
Maternal Surname	**First name**	**DoB**	**NHS Number**	**EDD**
Maternal Address		**GP Name and Address**		**PND reference** T
Maternity Unit		**Date of referral**	**Referrers name**	**PND result**

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PND OUTCOME FORM (Part 2) - Final Outcome

Part B to be completed within one month of delivery by Screening Co-ordinator or Specialist Nurse and returned to PND lab		**Outcome Form Unique number**: OXT	
Maternity Unit	**Date of referral**	**Name of Referrer**	**EDD**
Please return to *PND lab address*		**Please return by** *(one month from EDD)*	
Baby's NHS Number	**Newborn laboratory that baby's bloodspot was sent to**	**Baby's place of birth**	**If no live birth, please give reason**
Completed by (please print) NAME TELEPHONE		**Date** Part 2 B completed on	

Please complete parts in red, retain the named portion of this form (Part 2 A), and only return Part 2 B Final Outcome Form, with it's unique identifying number to the PND laboratory

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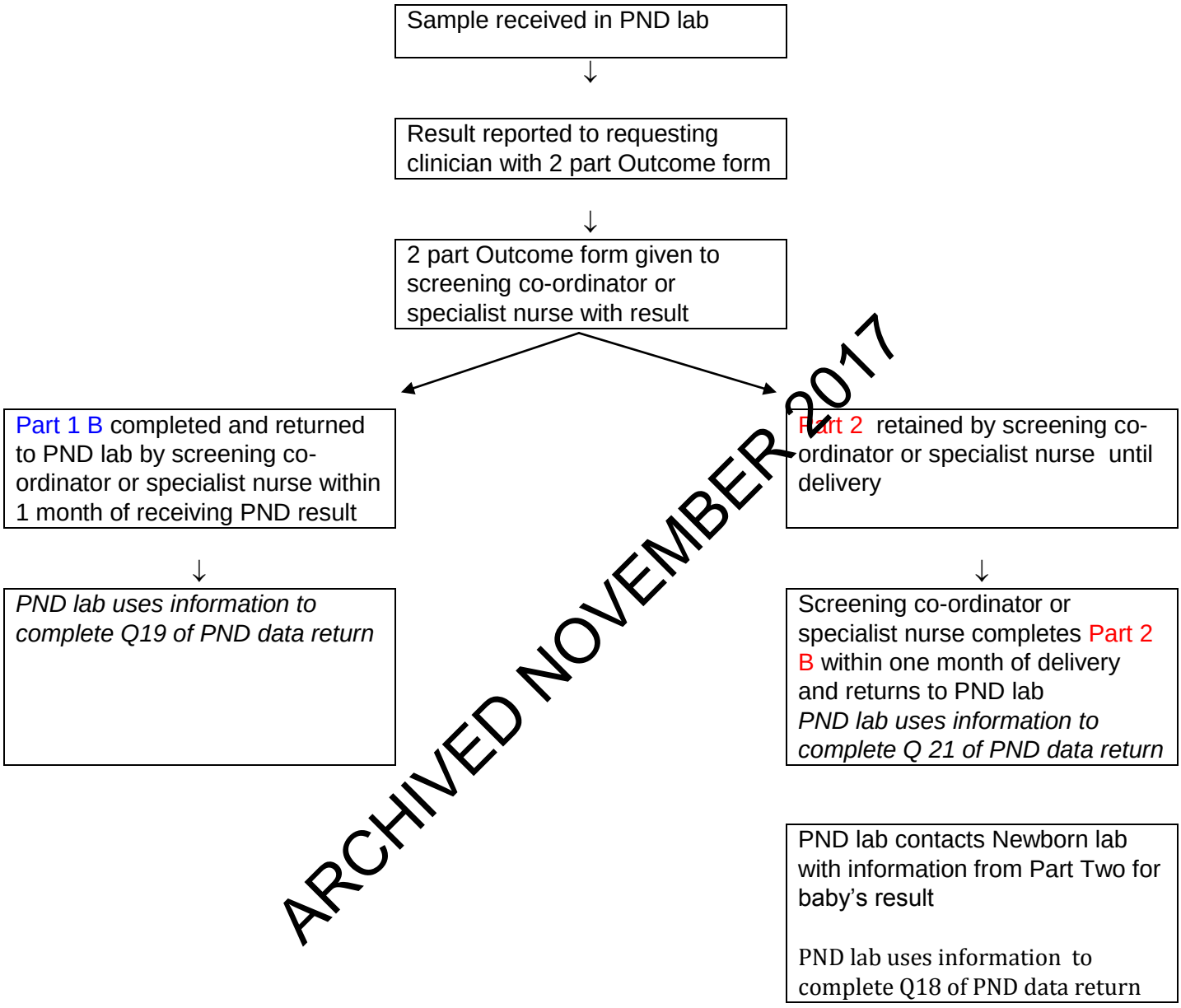
Flow Diagram for use of Two Part Pregnancy Outcome Form



The form should be sent be sent for all PND requests, not just those where PND shows an affected fetus.

The screening co-ordinator or specialist nurse is only required to complete parts in red and blue.

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Laboratory Contact details
Telephone: 020 3299 9000 Ext 2265 (lab)
Telephone: 020 3299 4337 (Office)
Fax: 020 3299 1035
Section Email kch-tr.PND@nhs.net

Denmark Hill
London SE5 9RS

Tel: 020 3299 9000
www.kingspath.co.uk
www.kch.nhs.uk

KCH REQUEST FOR PRENATAL DIAGNOSIS

	Mother's details	Partner's details
Surname		
Forename		
DOB		
NHS Number		
Ethnic Origin		
Genotype	For thal cases define mutation or state unknown.	Mutation (please tick one)
HbAS	(tick)	(tick)
HbAC	(tick)	(tick)
Beta thalassaemia		
Alpha thalassaemia		
Other		

Please Fax copies of all Haemoglobinopathy Screening Results to the laboratory (Fax no : 020 3299 1035)
Information in the table below is for UK referrals only.

Mother's Address:		GP Address:	
Post code:		Post code:	
Telephone:		Telephone:	

Fetal Sample type: CVS / AMNIO (please circle)

Maternal blood taken: Y/ N (please circle)

Gestation at sampling:…… ….
EDD or US EDD: ……
Date of sampling: ……

Paternal blood taken: Y/ N
Blood samples arriving with fetal sample Y/ N
Sampled at: HBR ☐ **GSTH** ☐ **Other** ……

Prenatal Diagnosis Report to be sent to:

PRIMARY REFERRER		COPY OF REPORT TO	
Name:		Name:	
Address:		Address:	
Tel:		Tel:	
Fax:		Fax:	

please note Fetal sampling will not take place AT THE HARRIS BIRTHRIGHT UNIT WITHOUT THE FOLLOWING:Hepatitas B / HIV / Rhesus status.
Results to be faxed to: HBR Unit: (Fax: 020 7733 9534) or Laboratory: (Fax: 020 3299 1035)

University College London Hospitals NHS
NHS Foundation Trust

For laboratory use only:
Date received……
Time received……
Laboratory number……

Haemoglobinopathy Genetics Centre
Ground Floor
86-96 Chenies Mews
London WC1E 6HX

Telephone: 0845 155 5000 Ext.75230
Direct Line: 020 3447 9458
Fax: 020 3447 9864
HaemGen@uclh.nhs.uk

UCH Request Form: Prenatal Diagnosis of Haemoglobin Disorders

	Mother's Details:	Partner Details:	Mother's GP Details:
Surname:			
First name:			
Date of birth:			N/A
Address:			
Post Code:			
Booking Hospital:			
Hospital No:			
NHS Number:			
Ethnic Origin:			
Parental Genotype:			
Any other relevant clinical information			

	Referrer:	Report to:	Invoice to:
Name:			
Hospital:			
Address:			
Post Code:			
Tel No.			
Fax No.			

Fetal Sample

Samples sent (please circle)	Maternal blood / Paternal blood / CVS / CVS DNA / Amniotic fluid / Fetal blood / Other (specify)		
Sampling method (please circle)	Transabdominal CVS, Transvaginal CVS, Amniocentesis, Fetal blood sampling		
Fetal Gestation	LMP:	EDD:	Age at Sampling:
Cytogenetic Lab Name		Cytogenetic Lab Tel No.	
Backup cultures in Progress (please circle)	Y / N	Cytogenetic cleaning carried out (please circle)	Y / N

Sample and Information Requirements

Fetal samples: CVS samples should be sent in an isotonic transport medium such as culture medium. Amniotic fluid samples: at least 10mls should be sent. Fetal blood samples must be sent in EDTA tubes.

Parental samples: Blood samples should be sent with each prenatal diagnosis request. 10ml of EDTA blood labelled with patient's surname, first name, DOB, Hospital number and the date of sampling.

Information: Please provide full Blood Counts and HPLC / Hb Electrophoresis results for the patient, partner, and other relatives where applicable. Please complete ethnic origin form or give ethnic origin above.

Please send samples in appropriate packaging, with completed signed request form, patient information and copy of consent form, to the above address by guaranteed post or courier.

Please sign below to confirm that the patient and partner have given appropriate consent for:

- Testing
- Samples will be stored
- Made anonymous and used as controls and test development

Signed:……..**Date:**……..

Ethnic Origin Form

(To be completed by the referring health professional)

		Patient	Baby's Father
A)	**MIXED**	☐	☐

Further information:.....

(B)	**WHITE**		
English, Scottish, Welsh or Irish		☐	☐
Other North European		☐	☐
Any other White background.....			
(C)	**MEDITERRANEAN**		
Greek or Greek Cypriot		☐	☐
Turkish or Turkish Cypriot		☐	☐
Italian, Maltese		☐	☐
Any other Mediterranean background.....			
(D)	**ASIAN**		
Indian or African-Indian		☐	☐
Pakistani		☐	☐
Bangladeshi		☐	☐
Any other Asian background.....			
(E)	**SOUTH EAST ASIAN**		
Chinese		☐	☐
Japanese		☐	☐
Thai, Vietnamese or Filipino		☐	☐
Malaysian or Indonesian		☐	☐
Any other SE Asian background.....			
(F)	**BLACK**		
African		☐	☐
Caribbean		☐	☐
Any other Black background.....			
(G)	**ARABIC**		
Arab African		☐	☐
Iranian		☐	☐
Iraq		☐	☐
Kurdish		☐	☐
Any other Arabic background.....			
(H)	**DON'T KNOW**	☐	☐

NATIONAL HAEMOGLOBINOPATHY REFERENCE LABORATORY

Director: Dr John Old, FRCPath.
Deputy Director: Dr. Shirley Henderson. PhD.
Sample reception: 01865 572769 Sec: 01865 572826 Fax: 01865 572775
Email: molhaem@ouh.nhs.uk. Website: www.ouh.nhs.uk/molhaem

Molecular Haematology
Level4
John Radcliffe Hospital
Oxford, OX3 9DU

REQUEST FORM: for Prenatal Diagnosis of Haemoglobin Disorders

Mother's details:		Partner's details:	
Surname:		Surname:	
Forename:		Forename	
Date of birth:		Date of birth:	
Address :		Address	
NHS number:		NHS number:	
Hosp No:		Hosp No:	

GP Name and address:	

Referred by:		**Report to:**	
Name		Name	
Address :		Address	
Telephone		Telephone	

INVOICE to be sent to:

Fetal Sampling Details

Indication of risk…… **Mother's genotype**…… **Father's genotype**……

Type of samples sent (please circle) : Maternal blood / Paternal blood / CVS / CVS DNA / Amniotic fluid / Fetal Blood

Date/time of fetal sample…… **Gestation at sampling:**,……

Cytogentics lab used for cleaning /culturing::……

Sample and Information Requirements (full details are in our "information for users" guide)

Fetal samples: The sample must be sent to a cytogenetics lab for cleaning /culturing before forwarding to us.
Parental samples: Fresh EDTA blood samples & FBC/HPLC results should be sent with the PND request form.
User Information: http://www.oxfordradcliffe.nhs.uk/forpatients/departments/labs/haematology/molhaem/haemoglobinopathies.aspx

Please telephone sample reception to book in the fetal sample before sending.
Please indicate to confirm if the mother and partner have given consent for their DNA to be stored and used in research and development projects that have been granted ethical approval (please delete as appropriate): Yes / No

Signed…… **Date**……

ETHNIC ORIGIN FORM

To be completed by the referring health professional

	Patient	Baby's Father
A. MIXED		
Further information _____		
B. WHITE		
English, Scottish, Welsh or Irish	☐	☐
Other North European	☐	☐
Any other white background_____		
C. MEDITERRANEAN		
Greek or Greek Cypriot	☐	☐
Turkish or Turkish Cypriot	☐	☐
Italian, Maltese	☐	☐
Any other Mediterranean background _____		
D. ASIAN		
Indian or African-Indian	☐	☐
Pakistani	☐	☐
Bangladeshi	☐	☐
Any other Asian background _____		
E. SOUTH EAST ASIAN		
Chinese	☐	☐
Japanese	☐	☐
Thai, Vietnamese or Filipino	☐	☐
Malaysian or Indonesian	☐	
Any other SE Asian background _____		
F. BLACK		
African	☐	☐
Caribbean	☐	☐
Any other black background _____		
G. ARABIC		
Arab African	☐	☐
Iranian	☐	☐
Iraq	☐	☐
Kurdish	☐	☐
Any other Arabic background _____		
H. DON'T KNOW	☐	☐