Fondazione Eqas Italia

EQA - External Quality Assessment
UK NEQAS
for Leucocyte Immunophenotyping
for General Haematology
for Blood Coagulation
for Blood Transfusion Laboratory Practice
for Feto-Maternal Haemorrhage
for Haematinics

Newsletter

Brescia, 16 Dicembre 2014

Carissimi colleghi,

facendo seguito alle numerose richieste di informazione ricevute a seguito di quanto riferito durante la sessione di ematologia dello 7° UK NEQAS Users Meeting, svoltosi a Milano lo scorso 13 Novembre, vi specifico le proposte di UK NEQAS for General Haematology per quanto riguarda lo studio delle emoglobinopatie.

Ad oggi sono stati sviluppati tre servizi principali, aventi diversi gradi di partecipazione, così denominati:

- ABNORMAL HAEMOGLOBINS, Hb A2 / Hb F
- NEWBORN SICKLE SCREENING
- DNA FOR HAEMOBLOBINOPATHIES

Il programma "**ABNORMAL HAEMOGLOBINS, Hb A2 / Hb F**" - Accreditato CPA - riguarda lo screening e/o l'analisi quantitativa delle emoglobine A, A2, F, S e viene proposto agli iscritti con cadenza bimestrale. I campioni preparati da UK NEQAS sono accompagnati da informazioni cliniche che comprendono i dati dell'emocromo, età, sesso, gruppo etnico e condizione clinica dei pazienti in studio.

Per quanto riguarda questo primo servizio esistono tre livelli di partecipazione:

- solo screening per l'anemia falciforme (Sickle Cell) per i partecipanti che eseguono il test di solubilità o altre tecniche manuali;

- screening per l'anemia falciforme (Sickle Cell) e identificazione delle frazioni emoglobiniche;

- partecipazione completa (screening e identificazione delle frazioni emoglobiniche) unitamente al nuovo programma "**Liquid Newborn Samples**".

Quest'ultimo nuovo schema costituisce un'aggiunta allo schema Abnormal haemoglobins e propone una soluzione per quei laboratori ai quali viene richiesto lo screening neonatale su un campione di sangue intero capillare (puntura dal Calcagno). Potrebbe trattarsi di un neonato a rischio di drepanocitosi per il quale i genitori o il pediatra non vogliono aspettare l'esito dello "spot test".

Viene inviato sei volte all'anno un singolo campione pediatrico per screening di emoglobinopatie (siglato come campione LN). Per ora questi sono inviati separatamente dai campioni destinati agli esercizi "Abnormal Haemoglobins", ma il progetto prevede il consolidamento degli invii non appena l'organizzazione logistica lo consentirà. Anche i report per i campioni LN sono per ora separati, ma saranno aggiunti ai report complessivi AH non appena completato il nuovo "format" e aggiornato il sito WEB da parte del servizio informatico.

Il servizio "**NEWBORN SICKLE SCREENING**" - Accreditato CPA - per lo screening dell'anemia falciforme del neonato, propone dodici esercizi annuali. Ogni volta sono inviati 3 campioni, come gocce di sangue essiccate su carta, ognuno contenente una goccia di sangue da cordone ombelicale. Tali campioni sono adeguati per analisi mediante HPLC, IEF (focalizzazione isoelettrica), CE (elettroforesi capillare) e MS (spettroscopia di massa).

Viene richiesto di identificare ed eventualmente frazionare e quantificare la variante patologica dell'emoglobina, in rapporto ai propri valori locali di riferimento. Occorre la disponibilità ad eseguire un test di falcizzazione, qualora richiesto su alcuni campioni specificamente identificati.

Lo schema "**DNA FOR HAEMOBLOBINOPATHIES**" è destinato ai centri che eseguono l'identificazione delle mutazioni puntiformi per lo studio delle talassemia Alfa e Beta. I campioni sono forniti come DNA in tampone TE (Tris-EDTA) e sono accompagnati da informazioni cliniche sul caso: sesso, origini etniche del paziente e altri dati ematologici. I partecipanti possono registrarsi alla partecipazione completa o solo per le mutazioni dell'Alfa talassemia.

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Con piacere allego a questa mia comunicazione alcuni esempi di report, che vi potranno sicuramente essere utili per approfondire quanto sopra riferito.

Inutile ribadire che i programmi EQA/PT proposti da UK NEQAS non sono da considerarsi come semplici controlli di qualità, bensì come un servizio indirizzato alla valutazione esterna di qualità per la verifica della performance dell'interno processo operativo, fornito unitamente alla consulenza diagnostica operata da professionisti esperti in medicina di laboratorio e in sistemi di qualità.

Rimanendo a vostra disposizione per qualsiasi necessità, colgo l'occasione per porgervi i miei più sentiti auguri per le prossime festività natalizie.

Dr. Emilio Ascari
Referente scientifico UK NEQAS for General Haematology

UK NEQAS

Abnormal Haemoglobins Scheme

Distribution: 1405AH **Date:** 29 Sep 2014

Sickle Cell Screening

Laboratory:

Survey Contents:

Specimen **1405SS1** was from a Sickle Positive donation

Specimen **1405SS2** was from a Sickle Negative donation

Specimen **1405SS3** was from a Sickle Negative donation

Non Participation Penalty: 0

Specimen Quality

	1405SS1	1405SS2	1405SS3
Satisfactory	412	413	411
Unsatisfactory	2	1	3
You reported:	Satisfactory	Satisfactory	Satisfactory

Return Rate

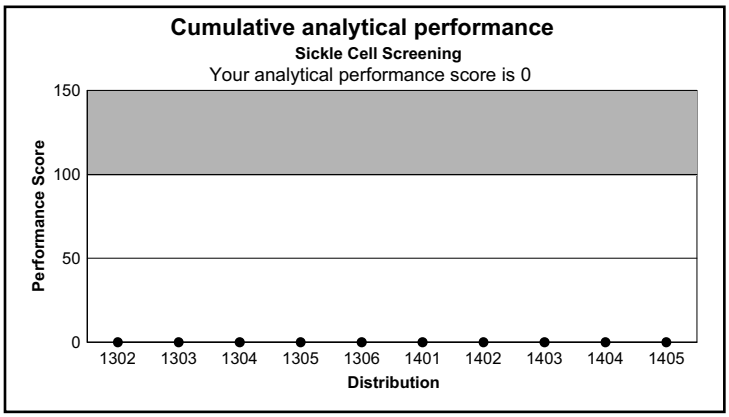
Specimens were distributed to 428 participants.
414 participants returned results.
This represents a 96% return rate.

Sickle Screen (Note: Neg = Negative; Pos = Positive)

Specimen	1405SS1		1405SS2		1405SS3	
	Neg	Pos	Neg	Pos	Neg	Pos
All Methods	10	384	393	2	390	4
Atlas Medical Sickle Cell Kit	0	1	1	0	1	0
Clin Tech	1	42	43	0	42	1
Coverslip reduction	1	15	16	0	15	1
DiaMed-ID Sickle Cell Test	0	1	1	0	1	0
HD Supplies	0	27	28	0	28	0
Helena Biosciences Europe HbS Solubility	0	11	11	0	11	0
Lorne	0	5	5	0	5	0
Microgen Bioproducts/S-Test/M96-50	1	76	76	1	77	0
Other - please specify	0	53	53	0	53	0
SA Scientific Sickle Cell Test	0	1	1	0	1	0
Solubility	6	39	45	0	43	2
Streck Sickledex	1	87	87	1	87	0
TCS Biosciences 'Sickle-Check Hb-S'	0	26	26	0	26	0

Your registered method	**Your Results**		
Coverslip reduction	**1405SS1**	**1405SS2**	**1405SS3**
	Positive	Negative	Negative
Sickle Screen Penalty	0	0	0

Cumulative Sickle Screen Analytical Performance Score	0



Participants are referred to the UK NEQAS (H) Participants' Handbook (version 6, April 2014; available to download from www.ukneqash.org) for an explanation of the performance monitoring system.

Comments:

Scheme Director: Professor K Hyde
UK NEQAS (H), PO Box 14, WATFORD WD18 0FJ, United Kingdom
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Fraction Identification & Quantification

Performance Score

Survey Contents:

Specimen **1405AH1** was from a Beta thalassaemia carrier donor

Specimen **1405AH2** Contained a mixture of normal adult blood and cord blood.

Specimen **1405AH3** was from a Sickle carrier donor (AS)

Non Participation Penalty: 0

Specimen Quality

	1405AH1	1405AH2	1405AH3
Satisfactory	310	311	310
Unsatisfactory	4	3	4
You reported:	Satisfactory	Satisfactory	Satisfactory

Return Rate

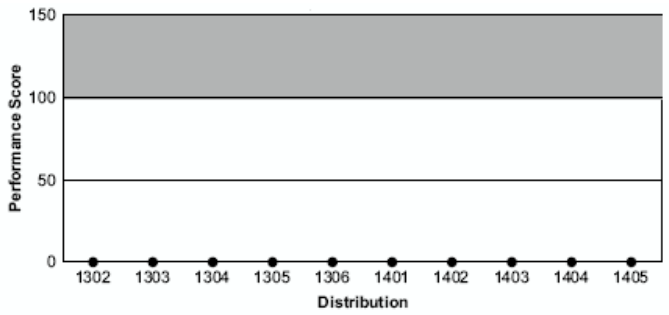
Specimens were distributed to 326 participants.
314 participants returned results.
This represents a 96% return rate.

PRN: 21762

Cumulative analytical performance

Your analytical performance score is 20.8

Haemoglobin A2 %

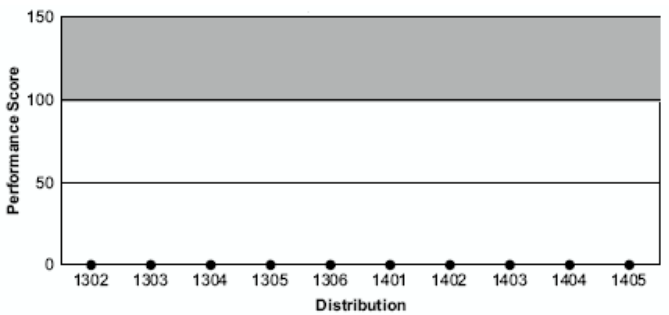


PRN: 21762

Cumulative analytical performance

Your analytical performance score is 19.5

Haemoglobin S %



Participants are referred to the UK NEQAS (H) Participants' Handbook (version 6, April 2014; available to download from www.ukneqash.org) for an explanation of the performance monitoring system.

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Fraction identification

Fraction	Expected	Essential	Your Results	Reported by all participants
Hb A	Expected	Essential	Present	301
Hb A2	Expected	Essential	Present	309
Hb F	Expected		Present	294
Hb S			Absent	0
Hb C			Absent	0
Hb D			Absent	0
Hb E			Absent	0
Hb C or E			Absent	0
Hb Non Specified Fraction			Absent	1

Performance summary for fraction identification

13 laboratories failed to report the fraction identification pattern essential for diagnosis.
Participants are asked to report all fractions present, including the expected ones (HbA, HbA2 & HbF).

Comments:
Twelve participants (all non-UK laboratories) failed to report the presence of Hb A. Four of these 12 laboratories also did not report the presence of Hb A2. One further laboratory did report Hb A and Hb A2, but also reported the presence of a non-specified fraction.

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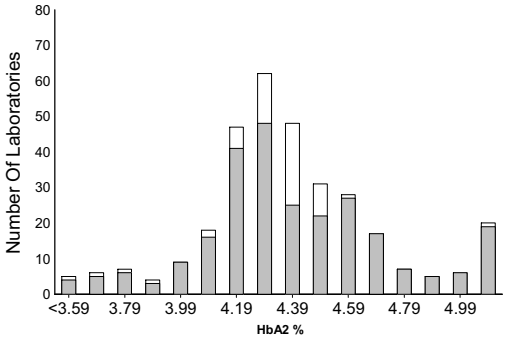
Fraction Quantitation

Haemoglobin A2 (%)

	n	Mean	GCV
All Methods	315	4.3	6.69
Capillary Electrophoresis	50	4.3	2.43
Sebia Capillarys	25	4.3	2.57
Sebia Capillarys 2	16	4.3	1.79
HPLC	255	4.3	7.51
Arkray HA8160	37	4.1	7.32
BioRad D10; Dual Program Kit	29	4.4	4.04
BioRad Variant Classic	11	4.2	2.91
BioRad Variant II; Beta-thal short pro	62	4.2	2.85
BioRad Variant II; Dual program Kit	24	4.4	4.60
Primus Ultra 2	20	4.2	3.00
TOSOH G7	22	4.9	7.99
TOSOH G8	38	4.6	5.47



Your registered method is:
HPLC BioRad D10; Dual Program Kit

Your Result :
DI :
Perf Score :

Reported Range (Overall)

Minimum	2.50
Maximum	5.80

Assessment vs your ref range

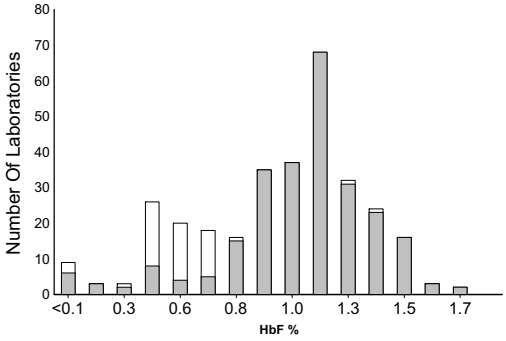
You reported:	High

Overall Assessment (%)

Low	
Normal	1.9
High	98.1
Uncertain	

Haemoglobin F (%)

	n	Mean	GCV
All Methods	300	1.00	44.44
Capillary Electrophoresis	48	0.60	19.14
Sebia Capillarys	24	0.60	17.26
Sebia Capillarys 2	15	0.60	11.78
HPLC	248	1.1	28.60
Arkray HA8160	39	1.1	20.01
BioRad D10; Dual Program Kit	29	1.1	11.47
BioRad Variant Classic	11	0.80	31.93
BioRad Variant II; Beta-thal short pro	62	0.90	11.15
BioRad Variant II; Dual program Kit	24	1.3	13.09
Primus Ultra 2	12	0.50	86.69
TOSOH G7	22	1.4	9.60
TOSOH G8	38	1.2	5.53



Your registered method is:
HPLC BioRad D10; Dual Program Kit

Your Result :

Reported Range (Overall)

Minimum	0.00
Maximum	1.70

Assessment vs your ref range

You reported:	High

Overall Assessment (%)

Low	0.3
Normal	75.2
High	23.2
Uncertain	1.3

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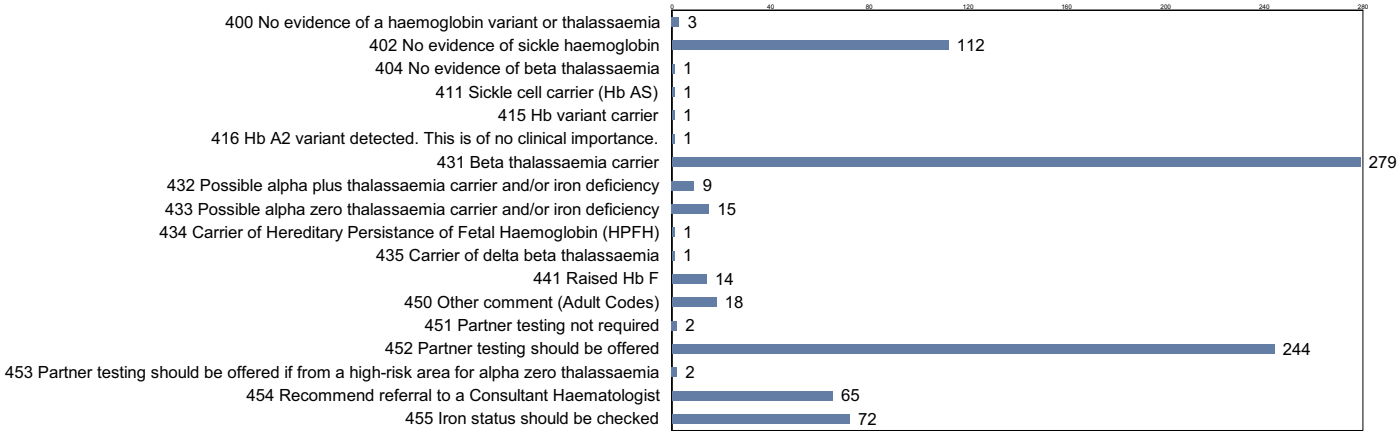
Specimen : 1405AH1

Interpretation

Sex	Female	RBC (10^{12}/L)	5.45
Ethnic Origin	Turkish	Hb (g/L)	119
Age	29 years	MCV (fL)	66.4
	Antenatal Screening	MCH (pg)	21.8

Analysis of Interpretation Codes reported by Participants



Data Analysis

Top five reported comments (see graph for all reported comments)

Code	Comment	Rank	Number
431	Beta thalassaemia carrier	1	279
452	Partner testing should be offered	2	244
402	No evidence of sickle haemoglobin	3	112
455	Iron status should be checked	4	72
454	Recommend referral to a Consultant Hae	5	65

Reported Comments

Your reported comments with the number of participants that reported the same comment

Code	Comment	Rank	Number
431	Beta thalassaemia carrier	1	279
452	Partner testing should be offered	2	244

Comments:

Specimen 1405AH1 simulated a specimen from a 29 year old Turkish female attending for Antenatal Screening. Her results showed a raised Hb A2 and, with the microcytic indices given in the FBC data, were consistent with a beta thalassaemia carrier.

279 of the participants who returned results by the closing day interpreted the results as a beta thalassaemia carrier; only 244 of those (87%) commented that partner testing should be offered.

One participant commented that there was no evidence of beta thalassaemia trait. A further participant commented that the patient was a sickle cell carrier, had not identified the presence of sickle haemoglobin in the fraction identification exercise, but reported 30.7% HbS on the results proforma. This participant may have transposed specimens or results. Two participants stated that partner testing was not required- one UK and one non-UK laboratory. The non-UK laboratory had reported a quantitative value for HbA2 within consensus but had not commented that the subject was a beta thalassaemia carrier.

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Fraction identification

Fraction	Expected	Essential	Your Results	Reported by all participants
Hb A	Expected	Essential	Present	301
Hb A2	Expected		Present	307
Hb F	Expected	Essential	Present	308
Hb S			Absent	0
Hb C			Absent	0
Hb D			Absent	0
Hb E			Absent	0
Hb C or E			Absent	0
Hb Non Specified Fraction			Absent	2

Performance summary for fraction identification

14 laboratories failed to report the fraction identification pattern essential for diagnosis.
Participants are asked to report all fractions present, including the expected ones (HbA, HbA2 & HbF).

Comments:

Four participants did not report the presence of Hb A or Hb F and eight laboratories reported the presence of Hb F but not Hb A. One laboratory did not report the presence of Hb F but did report a non-specified fraction and a further laboratory reported the presence of a non-specified fraction. All of these were non-UK laboratories.

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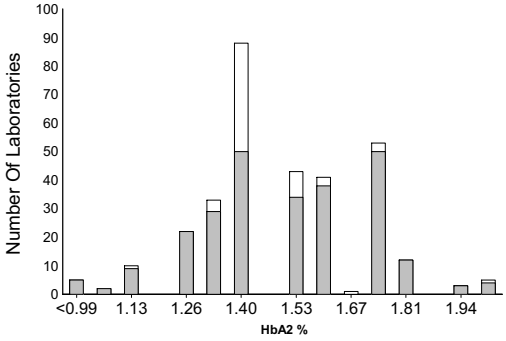
Fraction Quantitation

Haemoglobin A2 (%)

	n	Mean	GCV
All Methods	313	1.5	14.40
Capillary Electrophoresis	50	1.4	2.97
Sebia Capillarys	25	1.4	1.20
Sebia Capillarys 2	16	1.4	2.04
HPLC	253	1.5	16.23
Arkray HA8160	37	1.4	21.22
BioRad D10; Dual Program Kit	29	1.5	9.19
BioRad Variant Classic	11	1.7	2.84
BioRad Variant II; Beta-thal short pro	62	1.7	4.12
BioRad Variant II; Dual program Kit	24	1.4	11.29
Primus Ultra 2	20	1.4	5.18
TOSOH G7	22	1.4	11.09
TOSOH G8	38	1.2	9.35



Your registered method is:
HPLC BioRad D10; Dual Program Kit

Your Result :

DI :

Perf Score :

Reported Range (Overall)

Minimum	0.40
Maximum	2.30

Assessment vs your ref range

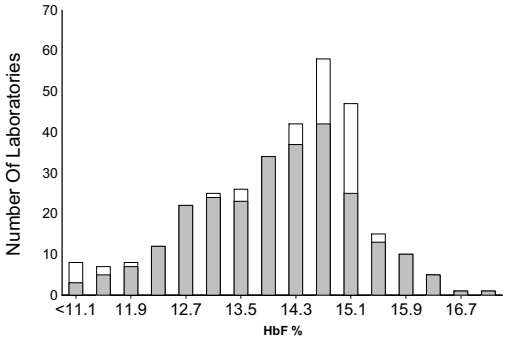
You reported:	Low

Overall Assessment (%)

Low	70.5
Normal	28.9
High	0.3
Uncertain	0.3

Haemoglobin F (%)

	n	Mean	GCV
All Methods	316	13.9	8.79
Capillary Electrophoresis	49	14.4	5.46
Sebia Capillarys	24	14.3	7.16
Sebia Capillarys 2	16	14.7	2.06
HPLC	259	13.9	8.74
Arkray HA8160	39	12.9	10.99
BioRad D10; Dual Program Kit	29	14.8	2.64
BioRad Variant Classic	11	13.4	14.42
BioRad Variant II; Beta-thal short pro	63	13.4	5.66
BioRad Variant II; Dual program Kit	24	15.2	5.63
Primus Ultra 2	20	13.4	8.24
TOSOH G7	22	13.6	5.01
TOSOH G8	39	14.4	3.47



Your registered method is:
HPLC BioRad D10; Dual Program Kit

Your Result :

Reported Range (Overall)

Minimum	4.20
Maximum	32.10

Assessment vs your ref range

You reported:	High

Overall Assessment (%)

Low	
Normal	
High	99.4
Uncertain	0.6

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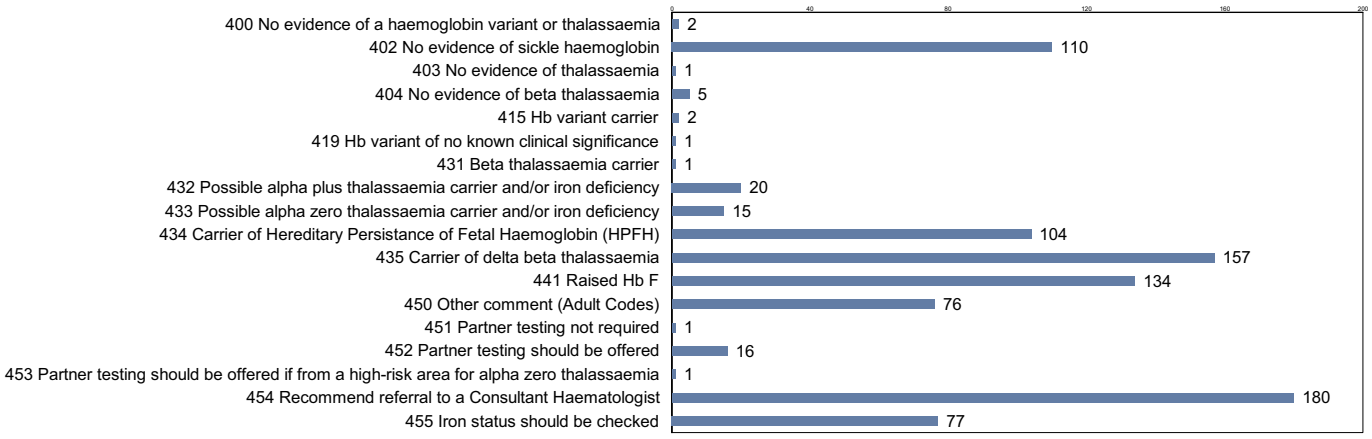
Interpretation

Sex	Male	RBC	(10^{12}/L)	5.01
Ethnic Origin	Eastern European	Hb	(g/L)	122
Age	31 years	MCV	(fL)	74.1
	Partner of pregnant lady who has sickle cell trait	MCH	(pg)	24.4

Analysis of Interpretation Codes reported by Participants



Data Analysis

Top five reported comments (see graph for all reported comments)

Code	Comment	Rank	Number
454	Recommend referral to a Consultant Hae	1	180
435	Carrier of delta beta thalassaemia	2	157
441	Raised Hb F	3	134
402	No evidence of sickle haemoglobin	4	110
434	Carrier of Hereditary Persistance of Feta	5	104

Report Comments

Your reported comments with the number of participants that reported the same comment

Code	Comment	Rank	Number
454	Recommend referral to a Consultant Hae	1	180
435	Carrier of delta beta thalassaemia	2	157

Comments:

Specimen 1405AH2 simulated a specimen from an Eastern European male whose partner was pregnant and has sickle cell trait. His results showed a raised Hb F and normal Hb A2, which, in conjunction with the reduced red cell indices given were consistent with a case of delta beta thalassaemia carrier.

Of the participants who returned a result by closing day, 157 participants returned a result of delta beta thalassaemia trait. 180 of participants indicated that a referral to a Consultant Haematologist was required. 104 participants reported the subject as a carrier for Hereditary Persistence of Fetal Haemoglobin.

One participant reported the case as a beta thalassaemia trait, but also included several other options for interpretation. Two participants reported the case as a carrier for a haemoglobin variant, both of whom stated that they would recommend a further sample to check. One participant reported as a haemoglobin variant of unknown clinical significance although they had accurately quantitated the raised HbF level.

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Fraction identification

Fraction	Expected	Essential	Your Results	Reported by all participants
Hb A	Expected	Essential	Present	300
Hb A2	Expected		Present	290
Hb F	Expected		Present	246
Hb S	Expected	Essential	Present	297
Hb C			Absent	0
Hb D			Absent	0
Hb E			Absent	0
Hb C or E			Absent	0
Hb Non Specified Fraction			Absent	5

Performance summary for fraction identification

23 laboratories failed to report the fraction identification pattern essential for diagnosis.
Participants are asked to report all fractions present, including the expected ones (HbA, HbA2 & HbF).

Comments:

Five participants did not report the presence of Hb A or Hb S; one of these participants reported a non-specified (NS) fraction. Eleven participants did not report the presence of Hb S. Of these 11 participants, 4 reported a non-specified (NS) fraction present. Four of the eleven who did not report Hb S were UK clinical laboratories: one reported a NS fraction and would refer the specimen to another site for identification; a further three did not indicate the presence of Hb S on fraction identification, but reported an Hb S percentage and gave the interpretation of sickle cell trait.
Seven participants did not report the presence of Hb A.

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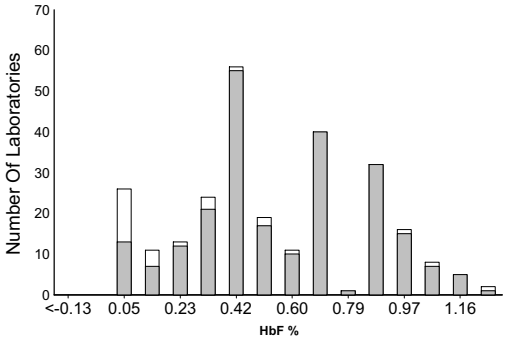
Fraction Quantitation

Haemoglobin F (%)

	n	Mean	GCV
All Methods	235	0.50	69.06
Capillary Electrophoresis	11	0.20	139.12
HPLC	220	0.50	64.60
Arkray HA8160	39	0.40	53.24
BioRad D10; Dual Program Kit	16	0.50	124.66
BioRad Variant II; Beta-thal short pro	61	0.40	20.97
BioRad Variant II; Dual program Kit	23	0.70	16.61
TOSOH G7	22	0.90	14.15
TOSOH G8	38	0.70	7.52



Your registered method is:
HPLC BioRad D10; Dual Program Kit

Your Result :

Reported Range (Overall)

Minimum	0.00
Maximum	1.70

Assessment vs your ref range

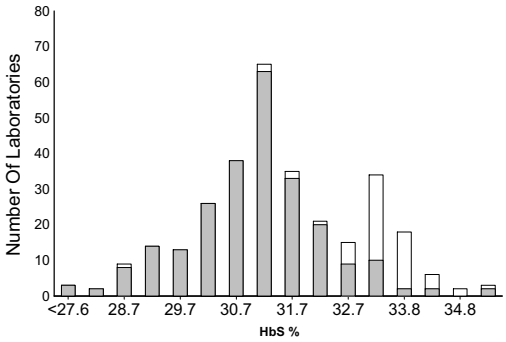
You reported:	Normal

Overall Assessment (%)

Low	2.8
Normal	95.2
High	2.1
Uncertain	

Haemoglobin S (%)

	n	Mean	GCV
All Methods	300	31.3	4.88
Capillary Electrophoresis	50	33.3	1.57
Sebia Capillarys	25	33.1	1.31
Sebia Capillarys 2	16	33.3	1.52
HPLC	241	30.8	3.67
Arkray HA8160	33	31.9	2.04
BioRad D10; Dual Program Kit	27	30.2	2.94
BioRad Variant Classic	10	30.9	2.48
BioRad Variant II; Beta-thal short pro	59	30.9	2.11
BioRad Variant II; Dual program Kit	22	30.4	3.05
Primus Ultra 2	19	31.1	0.99
TOSOH G7	22	30.1	6.16
TOSOH G8	37	30.6	5.13



Your registered method is:
HPLC BioRad D10; Dual Program Kit

Your Result :
DI :
Perf Score :

Reported Range (Overall)

Minimum	27.40
Maximum	36.50

Scheme Director: Professor K Hyde
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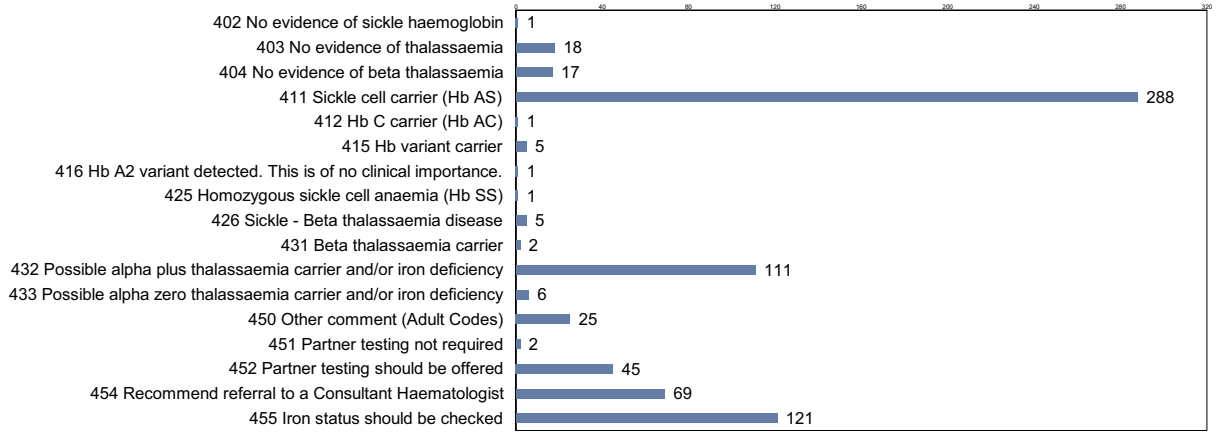
Specimen : 1405AH3

Interpretation

Sex	Female	RBC (10^{12}/L)	3.99
Ethnic Origin	Nigerian	Hb (g/L)	105
Age	49 years	MCV (fL)	81.2
	cause of low Hb level ?	MCH (pg)	26.3

Analysis of Interpretation Codes reported by Participants



Data Analysis

Top five reported comments (see graph for all reported comments)

Code	Comment	Rank	Number
411	Sickle cell carrier (Hb AS)	1	288
455	Iron status should be checked	2	121
432	Possible alpha plus thalassaemia carrier	3	111
454	Recommend referral to a Consultant Hae	4	69
452	Partner testing should be offered	5	45

Reported Comments

Your reported comments with the number of participants that reported the same comment

Code	Comment	Rank	Number
411	Sickle cell carrier (Hb AS)	1	288
452	Partner testing should be offered	5	45
450	Other comment (Adult Codes)	6	25

Comments:

This specimen simulated a sample from a 49 year old Nigerian female, referred for the investigation of a low haemoglobin level. The haemoglobin level given was reduced, as was the MCH and the results indicated the presence of sickle cell trait with possible co-existing alpha thalassaemia trait.

288 of the participants who returned results by the closing day gave an interpretation of sickle cell carrier and 111 (38%) commented on the possibility of the subject also being a carrier for alpha plus thalassaemia and / or having iron deficiency.

69 participants (24%) said they would recommend referral to a Consultant Haematologist.

Five laboratories interpreted the case as Hb S/beta thalassaemia. One participant reported it as Hb SS, one as a Hb C carrier, 2 as beta thalassaemia trait and one as having no evidence of sickle haemoglobin.

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UK NEQAS ABNORMAL HAEMOGLOBINS HbA_2, HbF and HbS SCHEME

Survey 1406LN: Liquid Newborn Samples (17th September 2014)

SUMMARY REPORT

Specimens distributed:

One specimen was distributed as a liquid blood sample from a newborn. The clinical information sent to assist with interpretation of results was:

- *Nigerian; male; Age: 10 hours old; RBC (10^{12}/L) 6.0; Hb (g/L) 112; MCV (fL) 105; MCH (pg) 30*

Sample 1406LN is from a 10 hour old male infant. Both the baby's mother and father are from Nigeria, neither have had haemoglobinopathy testing. The mother declined antenatal screening tests. The sample has been sent for haemoglobinopathy analysis.

Results returned:

31/32 participants (97%) returned results by the closing date.

Participant laboratories:

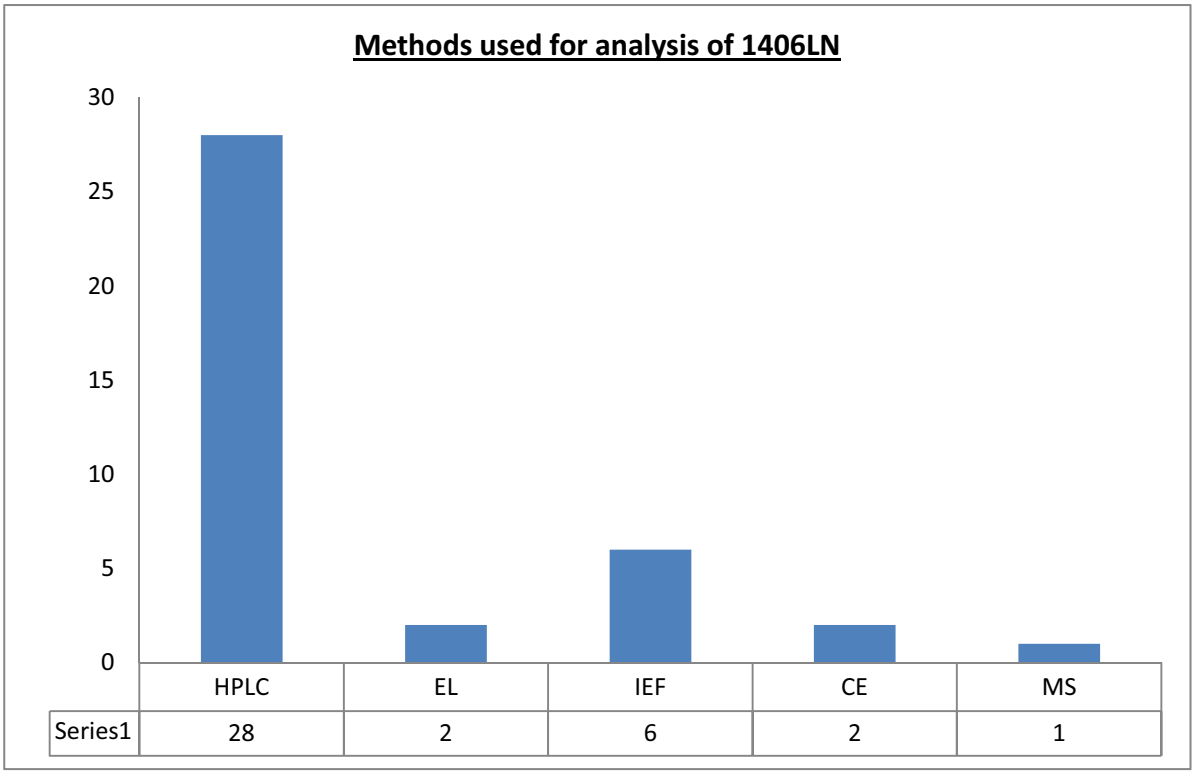
The participant base included UK registered laboratories only.

Specimen quality indicated by participants:

Specimen	N	Satisfactory	Unsatisfactory	Not stated
1406LN	31	31	0	0

Methods used to test 1406LN:

HPLC = High Performance Liquid Chromatography; EL= Electrophoresis; IEF = Isoelectric Focusing



Note: Some participants used more than one method in practice.

For information on data analysis and performance assessment see the UK NEQAS (H) Participants' Manual (www.ukneqash.org)

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Fraction identification results:

	1406LN1
Consensus result	**Hb F, Hb A, Hb S**
Laboratories giving consensus result	31
Out of consensus result	1
No result given	0

Coded comments returned:

Only the comments from those laboratories that returned the consensus fraction identification are included in the analysis for scoring, although the comments are all reported here.

Code	Expansion of code	Number of laboratories
		1406LN1
710	Results consistent with sickle cell carrier	30
745	Follow up referral required	4
750 [1]	Free text comments	17
Total number of returns		32

Notes:

[1] **The general free text code 750 comments concerned:**

- Refer for genetic counselling.
- HbS percentage lower than expected. Suggest repeat at 12 months.
- Suggest repeat in six months when adult haemoglobins have developed to confirm.
- The relative proportion of HbA: HbS is more consistent with A+S α^+ thal, but there is no Hb Barts evident on the chromatogram. Samples should be requested from both parents and any future pregnancy being at risk of producing a foetus with sickle cell disease until their screening results have been confirmed (postnatal if they refuse being screened).
- Hb A and S values not typical of sickle carriers. Possibly transfused? Suggest repeat at 3-6months of age.
- Valid if not transfused.
- Repeat in 6 months.
- Suggest repeat at 3-6months to confirm sickle status.
- Sickle cell carrier.
- Low % HbS detected on secondary technique of acid plate by heavy application only.
- Sample sent to specialist centre for confirmation by mass spec. Copy to sickle/thalassaemia centre.
- Refer to haemoglobinopathy nurse counsellor. Suggest repeat after 6 months of age.
- Results valid if not transfused. Repeat testing at 6 months to confirm HbS.
- Suggest repeat in 6 months.
- Thalassaemia cannot be excluded at this age. Result only valid if not transfused.
- HbF is normal in neonates. Valid if not transfused.
- Result valid if not transfused.

Next distribution: Distribution 1407LN is scheduled for 12th November 2014.

For information on data analysis and performance assessment see the UK NEQAS (H) Participants' Manual (www.ukneqash.org)

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Participant Reference Number:		20028
	This survey	Cumulative for 3 surveys
Non participation score (inc. late return):	0	0
Analytical performance score:	0	0
Interpretation score:	0	0

Survey 1410NH Newborn Sickle Cell Screening 14 October, 2014)

Specimens distributed

Three specimens were distributed as dried blood spots prepared from umbilical cord blood:
1410NH1: Gestation 40/40, Birth weight 3290 g, White and Black Caribbean
1410NH2: Gestation 34/40, Birth weight 2670 g, Caribbean
1410NH3: Gestation 32/40, Birth weight 2320 g, Caribbean

Following consultation with the scheme's Scientific Advisory Group, just one spot is supplied per specimen to make best use of survey material, which is obtained from clinical sources and is therefore of limited availability. On occasion you may require additional material and you should ask for a further sample. Up to 7 days additional turnaround time will be allowed, providing you contact us before the closing date.

Results returned

30/31 participants (97%) returned results by the closing date. One laboratory did not receive the survey material in time and returned results after closing date.

Participant laboratories

The participant base includes 16 UK primary Screening laboratories: 13 in England, one in Scotland, one in Wales and one in Northern Ireland; other participants include laboratories in the UK and Europe.

Specimen quality indicated by participants

Specimen	N	Satisfactory	Unsatisfactory	Not stated	Your result
1410NH1	31	30	0	1	Satisfactory
1410NH2	31	28	0	3	Satisfactory
1410NH3	31	28	0	3	Satisfactory

Methods used to test the specimens
(UK Newborn Screening Laboratories' results shown in parentheses)

HPLC = High Performance Liquid Chromatography; IEF = Iso-Electric Focusing; CE = Capillary Electrophoresis; MS = Mass Spectrometry

Method	Specimen		
	1410NH1	1410NH2	1410NH3
HPLC only	18 (10)	5 (0)	5 (0)
HPLC, confirmed by IEF	5 (1)	18 (11)	18 (11)
HPLC, confirmed by MS	0	0	0
HPLC, confirmed by CE	1 (0)	1 (0)	1 (0)
IEF only	1 (1)	0	0
IEF, confirmed by HPLC	0	1 (1)	1 (1)
IEF, confirmed by CE	0	0	0
CE only	3 (2)	1 (0)	1 (0)
CE, confirmed by HPLC	0	1 (1)	1 (1)
CE, confirmed by IEF	0	1 (1)	1 (1)
MS only	2 (2)	0	0
MS, confirmed by HPLC	0	2 (2)	2 (2)
MS, confirmed by IEF	1 (0)	1 (0)	1 (0)
Primary screening method not stated	0	0	0
TOTAL	**31 (16)**	**31 (16)**	**31 (16)**

Your screening methods:

Specimen	1410NH1	1410NH2	1410NH3
Your primary method	HPLC	HPLC	HPLC
Your secondary method	~	~	~

For information on data analysis and performance assessment see the UK NEQAS (H) Participants' Manual (www.ukneqash.org)
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Fraction identification results (UK Newborn Screening Laboratories' results shown in parentheses)

	1410NH1	1410NH2	1410NH3
Consensus result	**Hb F, Hb A**	**Hb F, Hb A, Hb S**	**Hb F, Hb A, Hb C**
Primary screening:			
Laboratories giving consensus / result consistent with consensus	31 (16) [1]	31 (16) [1]	31 (16) [1]
Out of consensus result	0	0	0
No result given	0	0	0
Your primary screening result	FA	FAS	FAC
Secondary screening:			
Testing not indicated	19 (15) [1]	0	0
Testing not available	5 (0)	6 (0)	6 (0)
As consensus result	7 (1)	25 (16)	24 (15)
Out of consensus	0	0	1 (1) [2]
No response given	0	0	0
Your secondary screening result	~	~	~

Notes and comments on the fraction identification results returned:

1. One UK newborn screening laboratory reported 'other result: sickle cell disorder is not suspected'. This result is consistent with the expected result for this specimen in terms of their national screening protocol, which is not designed to identify carrier states.
2. One UK primary screening laboratory reported "FAS" for 1410NH3 for on secondary screening, however, they correctly reported FAC using primary screening method and interpreted the final result as FAC.

UK newborn screening laboratories:

16/16 (100%) UK Newborn Screening Laboratories returned the consensus result for all three specimens.

Coded comments returned (UK Newborn Screening Laboratories' results shown in parentheses)

Only the comments from those laboratories that returned the consensus fraction identification are scored for interpretation, although the comments are all reported here.

Code	Expansion of code	Number of laboratories		
		1410NH1	1410NH2	1410NH3
700	No common haemoglobin variant detected: beta-thalassaemia trait cannot be excluded	30 (15)	0	0
710	Results consistent with sickle cell carrier	0	31 (16)	0
711	Results consistent with Hb C carrier	0	0	31 (16)
745	Follow up referral required	0	6 (2)	5 (3)
750 [1]	Free text comments	1(1) [1]	3 (2)	3 (2)
Total number of returns		31 (16)	31 (16)	31 (16)
Your result		700	710	711

Notes

[1] The general free text code 750 comments concerned:

- Sickle cell disorder is not suspected. *This is consistent with the participant's national screening protocol.*
- Refer to HbO counsellor.
- Refer to specialist health visitor.
- Reported to specialist Hbopathy Nurse.

Next distribution: Distribution 1411NH is scheduled for the 18th November 2014.

UK NEQAS for General Haematology

EQA for DNA Diagnostics in the Haemoglobinopathies Scheme Survey 1401DN (18th March 2014)

INTERIM REPORT

Date of issue: 14.8.2014

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DNA Diagnostics in the Haemoglobinopathies Scheme

Organisation

This scheme is operated by the UK National External Quality Assessment Scheme for General Haematology (UK NEQAS (H)). The Scheme Director is Professor Keith Hyde and the Scheme Manager Mrs Barbara De la Salle.

The scheme is operated under the expert guidance of the UK NEQAS (H) Special Scientific Advisory Group and Steering Committee.

Objectives

The objectives of this scheme are to assess:

- The interpretation of haematological data (with salient clinical information) to direct appropriate DNA tests.
- The use of DNA investigations to achieve the correct results. The exercise was not intended to assess the techniques, methodology or approach to analysis used, except in their ability to achieve the expected results.

Distribution schedule

Distribution 1401DN was dispatched on 18th March 2014. 1402DN was dispatched on 9th July 2014. Unfortunately, due to sampling error, this survey had to be withdrawn. The replacement, designated '1402DNReplacement' will be distributed early in September. Participants will be notified of the dispatch by email. 1403DN is scheduled for distribution in November 2014.

Survey 1401DN (18th March 2014)

Survey material

Two specimens (1401DN1, 1401DN2) were distributed. The specimens were accompanied by background information that included gender, ethnic origin, age, full blood count and haemoglobinopathy screen data.

Specimen distribution and return of results

Specimens were distributed to the 45 laboratories registered for the scheme and 42 (93%) returned a results sheet. 9 UK laboratories were registered for this survey and all 9 returned a results sheet.

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1. ANALYSIS OF RESULTS FOR SPECIMEN 1401DN1

1.1 Expected results for specimen 1401DN1

Female, 30 yrs, Greek origin
Reason for referral: Investigation of abnormal results found on pre-operative checks

Laboratory Results for 1401DN1

Parameter	Result
Hb (g/L) RBC ($x10^{12}$/L) MCV (fL) MCH (pg)	107 5.2 66 20.4
Haemoglobinopathy screen	No abnormal haemoglobin detected
Hb A_2 (%)	5.3
Hb F (%)	0.4
DNA Analysis	Alpha globin genotyping: Alpha genotype: $\alpha\alpha/\alpha\alpha$ Beta globin genotyping: Beta genotype: $\beta^{A}/\beta^{IVSI\text{-}110(G>A)}$

1.2 Specimen quality

Of the 42 returns received, all participants reported sample 1401DN1 as satisfactory.

1.3 Methods used

Alpha genotype	Beta genotype
Multiplex Gap PCR (19)	Sequencing (31)
Gap PCR (8)	Vienna labs strip assay (6)
Sequencing (11)	MLPA (4)
Vienna labs strip assay (8)	Multiplex Gap PCR (1)
MLPA (9)	ARMS (1)
	DGGE (1)
	Reverse hybridisation after multiplex PCR (1)

Some laboratories use more than one method in combination.

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1.4 Alpha genotype results for specimen 1401DN1

35 participants undertook alpha thalassaemia genotyping. All 35 reported the expected genotype, αα/αα.

1.5 Beta genotype results for specimen 1401DN1

36 participants undertook beta genotyping.
34 reported the expected genotype $\beta^{A}/\beta^{IVS1\text{-}110(G>A)}$ (2 of these participants reported the genotype as β^{A}/β^{+} but gave the appropriate HGVS identification).
1 participant reported the genotype as β^{A}/β^{+} and did not give any HGVS identification.
1 participant reported the genotype as $\beta^{A}/\beta^{1\text{-}1(G>A)}$.

1.6 Interpretation of results for 1401DN1

Appendix1 contains a summary of the interpretive comments for 1401DN1.

1.7 Expert comment 1401DN1

To follow with finalised report.

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2. ANALYSIS OF RESULTS FOR SPECIMEN 1401DN2

2.1 Expected results for specimen 1401DN2

Female, 3 months, African origin

Please confirm the nature of the haemoglobinopathy present.

Laboratory Results for 1401DN2

Parameter	Result
Hb (g/L) RBC ($x10^{12}$/L) MCV (fL) MCH (pg)	109 4.4 77 24.8
Haemoglobinopathy screen	HPLC: Fractions found in HbS, HbC, and HbF windows. Small fraction also noted in the Hb A_2 position.
Hb A_2 (%)	1.3
Hb F (%)	51.1
DNA Analysis:	Alpha globin genotyping: Alpha genotype $-\alpha^{3.7}/\alpha\alpha$ Beta globin genotyping: Beta genotype: β^S/β^C

2.2 Specimen quality

Of the 42 returns received, all participants reported sample 1401DN2 as satisfactory.

2.3 Methods used

Alpha genotype	Beta genotype
Multiplex Gap PCR (21)	Sequencing (29)
Sequencing (11)	MLPA (4)
MLPA (8)	Vienna labs strip assay (6)
Gap PCR (11)	Multiplex Gap PCR (2)
Vienna labs strip assay (7)	ARMS (1)
	Gap PCR (1)
	Reverse hybridisation after multiplex PCR (1)
	PCR Digest (1)
	DGGE (1)
	HBB codon 6 specific real-time PCR assay (1)

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Some laboratories use more than one method in combination.

2.3 Alpha genotype results for specimen 1401DN2

35 participants undertook alpha genotyping on this specimen. All 35 reported the genotype as $-\alpha^{3.7}/\alpha\alpha$.

2.4 Beta genotype results for specimen 1401DN2

39 participants undertook beta genotyping, all 39 reported the expected genotype β^{S}/β^{C}.

2.5 Interpretation of results for 1401DN2

Appendix 2 contains a summary of the interpretive comments for 1401DN2.

2.6 Expert comment for 1401DN2

To follow with finalised report.

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	Sample identification: 1401DN1 Material: Analyses are performed on extracted DNA from UKNEQAS. Result: The patient is heterozygous for HBB:c.93-21G>A, and this mutation results in a beta plus thalassemia with some remaining expression of the affected allele (Reference: the HbVar database). The DNA-analyses performed could not detect any deletions or mutations causing alpha thalassemia or alpha globin variant. Interpretation: Beta thalassemia minor is usually consistent with a mild anaemia and a low MCV and MCH. This genetic trait can be transferred to future generations. The beta thalassemia trait (HBB:c.93-21G>A) can in combination with certain other genetic haemoglobin mutations result in severe disease. Genetic counselling may be required prior to having children. Methods used: MLPA, HB alfa-locus (MRC Holland) MLPA, HB non alpha locus (MRC Holland) Sanger sequence HBB (NM_000518.4) Sanger sequence HBA1 (NM_000558.3) Sanger sequence HBA2 (NM_000517.4)	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	Results: PCR testing for the common alpha gene deletions was normal. PCR testing for the triplication of alpha gene was normal. Beta Globin Sequencing NG_000007.3(HBB):c[93-21G>A] ; [315+26T>G] DNA sequencing of the beta-globin gene has detected a heterozygous, single base change (G>A) in IVS1 position 110. This mutation creates a new acceptor splice site, resulting in a Beta (+) Thalassaemia. In addition, DNA sequencing of the beta-globin gene has detected a heterozygous, single base change (T>G) in IVS2 position 26. **Interpretive Comment:** Alpha globin genotype: αα/αα Results consistent with Beta Thalassaemia trait. There is a reproductive risk of a severe thalassaemic syndrome when co-inherited with other beta globin mutations. The clinical significance of the second mutation (c.[315+26T>G]) identified in the beta globin gene is unknown. Partner testing, family studies and genetic counselling should be considered where appropriate.	
	Haematological indices indicated a heterozygous Beta Thalassaemia mutation and this was confirmed by sequencing. The IVS-I-110 (G->A) mutation is located 21 nucleotides 5' to the acceptor splice site AG^GC in intron 1. This mutation causes the formation of a new splice site resulting in an 80% abnormal spliced mRNA and 20% normal mRNA. 1401DN1 is heterozygous for Beta + Thalassaemia. The partner of 1401DN1 should be checked for Beta Thalassemia indices.	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	β^{+} Thalassaemia carrier. Genotype IVS-nI-110 G-A. If inherited with a severe Beta thalassaemia mutation, could result in a severe transfusion dependent Beta thalassaemia major phenotype. Partner testing advised. Genetic counselling recommended.	
	Beta thalassaemia trait. Heterozygous for the severe beta plus thalassaemia mutation IVS1-110 (G-A) [HBB:c.93-21G>A]. Negative results were obtained for non-deletional alpha plus thalassaemia and the common alpha plus thalassaemia deletion mutations.	
	None of the common alpha zero thalassaemia deletions (SEA, FIL, MED, THAI or 20.5) or alpha plus thalassaemia deletions (3.7 or 4.2) were detected by multiplex GAP PCR analysis. However, alpha thalassaemia due to non_deletional or very large deletions is not excluded. DNA analysis by Sanger sequencing confirms the mutation (HBB:c.93-21G>A) IVS~1~110 (G>A) beta +. This data is consistent with heterozygosity for Beta (+) thalassemia.	
	None of the common alpha thalassaemia deletions ($-\alpha^{3.7}$,$-\alpha^{4.2}$, $--^{SEA}$, $--^{THAI,}$ $--^{FIL}$, $--^{MED}$ and $-(\alpha)^{20.5}$) were detected in this patient. Beta globin gene analysis was not performed. RBC indices and the elevated HbA_2 value are suggestive of beta thalassaemia trait.	
	The common deletional types of alpha thalassemia are excluded. The patient is heterozygous for beta plus thalassemia ($\beta^{A}/\beta^{IVS\ I\ 110(G>A)}$). Parental testing and genetic counselling are recommended. The haematological phenotype suggest a regular patient follow up.	

For information on data analysis and performance assessment see the UK NEQAS (H) Participants' Manual (www.ukneqash.org)

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	Genotyping for the main deletions and mutations of the alpha-globin loci is normal. With the techniques used, we can not exclude the possibility of rare deletions or mutations. Taking into account these limitations, the patient has a full set of functional alpha-globin genes.	1401 DN1 is a heterozygous beta-thalassemia carrier, as suggested by the HbA2 value and the microcytosis. Parents should be advised that there is a ¼ risk of beta-thalassemia major for pregnancies if both are beta-thalassemia carriers.
	Heterozygous beta–thalassaemia $\beta^{A}/\beta^{IVS\text{-}I\text{-}110\ (G->A)}$ **HGVS:** NG_000007.3:g.70796G>A Appointment with hematologist should be offered. Partner testing should be offered. Iron testing should be offered.	
	Adult female with beta-Thal minor. In case of family planning the partner should be checked for any haemoglobinopathies to rule out eventual compound situations.	
	DNA investigation of the α-gene and β-gene locus revealed a base substitution in one of the β-genes that still allows some expression of β-globin from that locus. The β-gene is intact, as is the α-locus. The β-thalassaemia minor with modest impairment of beta-chain synthesis is characterized by mild microcytic anaemia. Carrier testing for haemoglobinopathies is recommended for the partner and first degree relatives.	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	The patient has CBC and high HbA2 compatible with β-thalassaemia minor and indeed she is Ht for IVSI-110G>A. No deletion or mutation was found in alpha globin. Genetic counselling should be provided.	
	Deletions - $\alpha^{3.7}$, - $\alpha^{4.2}$, -$(\alpha)^{20.5}$,-- ^{MED}, --^{SEA}, -- ^{FIL} and – ^{THAI} were not detected. The patient individual is a normal homozygous for the analysed deletions. Hematological results are indicative of beta thalassaemia trait. Genetic counselling should be offered.	Beta genotype is performed in laboratory participant reference number 29510.
	The patient is heterozygous for the following β-thalassaemia mutation $\beta^{A}/\beta^{IVS\text{-}1\text{-}110(G>A)}$ {HBB{NG_000007.3}:g.70796G>A). This mutation is associated with a weak residual expression of the mutated allele. The patient is therefore carrier of a β^{+} thalassaemia which is consistent with the result of the blood analysis. This mutation is frequently found in Mediterranean populations. The sequence of the beta gene shows a second abnormality: a T>G mutation at nucleotide 26 in Intron II. This mutation is considered as a polymorphism and is not reported in the protocol. MLPA analysis of the alpha globin cluster and sequencing of the α1 and α2 globin genes were negative for α-thalassaemia deletions and non-deletion mutations. Genetic counselling and partner testing are recommended.	
	No comments given	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	The result shows the mutation c.93-21G>A (IVS-I-110(G>A)) in the ß-globin gene in the heterozygous state. Due to the present genotype the patient is carrier of heterozygous ß(+)-thalassemia in combination with heterozygous α(+)-thalassemia leading to the phenotype of ß-thalassaemia minor in combination with asymptomatic α-thalassaemia. The resulting phenotype is mainly characterized by a slight change in the hematologic profile and usually presents as an asymptomatic mild hypochromic microcytic anemia. Offspring inherits the mutations with a risk of 50%. We recommend genetic counseling. The MLPA analysis showed no deletion in the α – and ß-globin gene complex. No mutations in the α-globin genes HBA1 and HBA2 were detected.	
	The patient is a beta-thalassemia carrier (heterozygous for HBB: c.92+110G>A mutation). The partner should be tested for hemoglobinopathies (hematological parameters and hemoglobinopathy screening). In the case of thalassemia and/or Hb variants genetic counselling and molecular analysis should be offered. Close relatives (parents, siblings) should be sent for hematological evaluation.	
	Heterozygous IVS 1.110G>A Alpha genes not tested. Beta Thalassaemia trait.	
	β-gene analysis: The sample was sequenced and found heterozygote for IVSI-110 (G>A) Genotype: $\beta^{A}/\beta^{IVSI\text{-}110(G>A)}$ α-gene analysis: The sample was also analysed by MLPA (α-locus) and α2 and α1 genes were sequenced. No mutations were detected. Genotype: $\alpha\alpha/\alpha\alpha$	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	The results obtained revealed that **1401DN1 patient is a carrier for the c.93-21G>A mutation in intron 1 of the HBB gene** (mutation previously named as $\beta^{IVS1\text{-}110G>A)}$. This mutation gives rise to a new splice site resulting in ~80% abnormal spliced mRNA and ~20% normal mRNA, being considered as a beta^{+} mutation (http://globin.cse.psu.edu/html).**The patient is a carrier of a severe beta+-thalassaemia, her genotype is consistent with her haematological and biochemical data (microcytosis, hypochromia and elevated Hb A2).**Close relatives of 1401DN1 patient (parents, siblings) should be sent for haematological evaluation; in case of thalassaemic indices, genetic counselling and molecular analysis should be offered. If the patient is married, her husband should be submitted to the same evaluation.Genetic counselling is also recommended to the patient and to her husband (if he is a carrier).	
	A heterozygous mutation was detected in the HBB gene, the patient is therefore carrier of beta-thalassemia. Genetic counselling for consultand and family members is indicated, carrier testing of the partner and first degree relatives is recommended.	
	Heterozygous carrier of severe β+ thalassemia. Genotype: $\beta^{A}/\beta^{IVS\text{-}I\text{-}110\ (G>A}$) or HBB:c.93-21G>A This mutation is very common in Greece. A homozygous or compound heterozygous constellation causes almost invariably a transfusion-dependent β thalassemia major. Genetic counselling and family testing is indicated. Partner testing is indicated before family planning.	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	Alpha Globin Gene (using MLPA): No deletions found in HBA1 and HBA2 region. **Beta Globin Gene (using fluorescent DNA sequencing):** Positive for the Mediterranean heterozygous IVS1-110(G>A) beta plus mutation [**HGVS name** HBB:93-21G>A] **Conclusion:** Beta thalassaemia trait. Genotype: IVS1-110 (G>A)/N. This is consistent with microcytosis and raised HbA2 result. No common deletional alpha thalassaemia found. In view of the Hb results suggest test ferritin. Genetic counselling recommended.	
	The patient is a heterozygous carrier of the beta+ mutation IVS1.110G>A in the HBB gene. This finding is consistent with the elevated HbA_2 and the reduced MCH and MCV values. Genetic counselling is recommended. Testing of family members can be offered.	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	Patient is heterozygous for the IVSI-110 G>A mutation (mutation beta+ severe). This genotype is in agreement with the haematological phenotype and with hemoglobin studies and corresponds to a phenotype of beta thalassemia minor. According to national regulations, genetic counselling and family screening should be offered to first degree relatives and to the proband's spouse.	For 1401DNA : we also identify a sequence variation of unknown significance (VUS) in intron 2 of HBB: IVS2 nt 26T>G (HGVS:c.315+26T>G). This sequence variation was not reported because the presence of the heterozygous IVS1-110G>A mutation explains the phenotype; this variant is unlikely to add valuable information at the clinical level and reporting could lead to misinterpretation.

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	Sequence analysis of the sample of genomic DNA from this individual has detected that she is heterozygous for the common c.93-21G>A¥ splice site mutation in intron 1 of the HBB gene. **She is therefore a carrier of beta+ thalassaemia and this is consistent with her red cell indices**. Further analysis using gap PCR to detect large deletions of the *HBA* genes has not identified the - $\alpha^{3.7, --MED, --FIL, --SEA}$ or -$(\alpha)^{20.5}$ deletions of the *HBA1* and *HBA2* genes. Other haemoglobin mutations, including non-deletion mutations have not been investigated. Other members of this individual's family are at risk of being affected with beta-thalassaemia and screening for this mutation is available. We recommend referral to your local Genetic Counselling Service where testing can be offered, if appropriate. DNA has been stored from this individual. If you require any further information on these results, please do not hesitate to contact the undersigned.	
	The hematological parameters of this woman indicate that she is a carrier of beta-thalassemia. We have found that she is heterozygote for the mutation IVS1-110 (HBB:c.93-21G>A). This mutation, although considered to be a b+ mutation, give a picture of severe phenotype. No triplication of alpha-globin gene was detected.It is recommended to advise the woman to check her partner, when relevant, and refer for genetic counselling.	
	Patient is heterozygous for HBB IVS 1.110 G>A corresponding to a heterozygous β^+ thalassaemia. This genotype is in concordance with results of the Hb-chromatography and haematological diagnostics.	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	Result: • Beta globin genotyping: **Heterozygous genotype: [c.93-21G>A];[=]** Methods: PCR amplification of the beta globin gene (NM_000518.4) and sequencing promoteur, exonx, introns and polyA sequence. • Alpha globin genotyping: **No mutation detected by the method used. Apha genotype: αα/αα** ***Methods: MLPA kit MRC Holland*** **Interpretation** **The patient is heterozygous for a beta-plus thalassaemia severe mutation (HBB:c.93-21G>A; also known as IVS-I-110 (G>A) ref HbVar) leading to a minor beta-thalassaemia** and results in mild anemia with low MCV as observed. There is a reproductive risk of a severe thalassaemic syndrome when co-inherited with other beta-haemoglobinopathies. Family screening and genetic counselling should be considered.	
	No results returned	
	Patient is carrier of a β+ severe thal trait. Genetic counselling should be proposed in order to discuss of the association of this trait with another beta trait in case of a pre-conceptional project.	
	Possible iron deficiency because Hb is quite low for a single β^+-thal mutation at the heterozygous state	

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	Haematological interpretation	Additional comment
	The patient is heterozygous for the following β mutation : β^{A} / $\beta^{IVSI\ 110G>A}$. This mutation creates a new acceptor splice site, resulting in a β^{+} thalassaemia. It is common in the greek population. Partner testing should be offered if any.	
	No results returned	
	Sample heterozygous for IVS I-1 (G>A) No analysis for a-thalassaemia	
	DNA analysis has shown that **1401DN1** is heterozygous for the β^{+} mutation IVSI-110 (G>A) (Beta plus thalassaemia trait). Any offspring of **1401DN1** have a 1 in 2 risk for inheritance of this mutation. When co-inherited with another beta thalassaemia mutation the resulting phenotype can range from moderately severe beta-thalassaemia intermedia to severe transfusion dependent thalassaemia major. In addition, **1401DN1** tested negative for the 7 most common forms of deletional alpha thalassaemia. **Conclusion:** **From the beta genotype identified in 1401DN1 we recommend screening of any current or future partner to determine the risk of having a child with a clinically significant haemoglobinopathy.**	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	INDICATION: Suspicion of a beta-thalassaemia trait. METHOD: PCR amplification followed by direct sequencing of HBB gene. Our reference sequence is the coding sequence NM_000518.4 (A from ATG =1) RESULT: Identification of the c.93-21G>A mutation in the heterozygous state (beta+). Identification of the c.315+26T>G variation in the heterozygous state. CONCLUSION: The heterozygous c.93-21G>A mutation confirms a beta-thalassaemia trait for your patient. We have also identified a intronic variant not yet described (c.315+26T>G); nevertheless we have already detected this variant in our patient's population and we have considered it as probably not pathogenic. A genetic counselling is recommended. N.B. Beta-thalassaemia intermedia is a clinical definition. If suspected, further analysis might be performed.	
	No comments given	
	Result: Mutation HBB:c.93-21G>A (IVS1-110) identified at the heterozygous state. c.93-21G>A is a β^+ mutation. Conclusion: This analysis conclude to a diagnostic of minor beta-thalassaemia. Your patient is a carrier for beta-thalassemia. A familial study is recommended. Testing the (future?) husband for haemoglobinopathy and a genetic consultation are recommended. Remark: This analsysis includes an investigation for sickle cell disease, Haemoglobinopathy	We do not use the " β^A/β^A " nomenclature in our reports. We use the HGVS nomenclature in our report and the accession number of the gene is given in our report in the "technical

APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	C and E. This investigation was negative in your patient.	session". We also keep the "ancient" nomenclature like E6V for sickle cell disease mutation in our report.
	Heterozygous beta^{0} Thalassemia minor type IVS 1.110 [G>A]	
	The supplied laboratory results prompted us to check for the presence of thalassemic aberrations. The beta-strip (ViennaLabs) revealed heterozygosity for the beta-plus mutation *HBB:*c.93-21G>A [alternative name: IVS-I-110 (G>A)]. We additionally checked for common deletions in the HBA gene cluster using gap-PCR (deletions tested for: 3.7, SEA, MED, 20.5, FIL, and THAI). None of the mentioned deletions was detected. Accordingly, 1401DN1 is a carrier for a beta-plus mutation, which clinically expresses as beta-thalassemia minor. We recommend testing the family and potentially the patient's partner (in case of family planning). These results should be communicated in the context of a genetic counselling.	We don't use the $\alpha\alpha/\alpha\alpha$ and β^{A}/β^{A} nomenclature in our reports. Poorly designed result sheet.

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	NEQAS 1401DN1 is heterozygous for beta plus thalassaemia due to the IVSI-110 (G >A) mutation (Beta thalassaemia carrier). The sample is also negative for the common alpha thalassaemia deletions. These include the alpha + thalassaemia 3.7 kb and 4.2 kb deletions and the alpha 0 thalassaemia SEA, MED and 20.5 kb deletions. These genetic results are consistent with the phenotypic findings. If considering starting a family partner testing recommended. DNA used in this diagnosis has been stored indefinitely. If you have any queries concerning this result, please do not hesitate to contact the laboratory.	

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APPENDIX 1: Interpretive comments for 1401DN1

	Haematological interpretation	Additional comment
	THIS IS AN UPDATED AND FINAL REPORT CARRIER OF BETA THALASSAEMIA (BETA IVSI-110(G>A) also known as HBB:c.[93-21G>A]+[=] ALPHA THALASSAEMIA OF COMMON TYPES (SEE DETAILS BELOW) TESTED FOR AND NONE DETECTED This result indicates the patient is a CARRIER of serious inherited Haemoglobin Disorders. It should cause the patient no harm provided specific tests for iron deficiency are carried out if needed, but has implications for other members of the patient's family. INFORMATION AND GENETIC COUNSELLING must be offered to the patient and their family. Referred for investigation of abnormal results found on pre-operative checks. Full blood count and haemoglobin disorders' screen showed a beta thalassaemia carrier state, referred for DNA studies. Beta globin gene sequence analysis showed heterozygosity for HBB:c.93-21G>A (beta plus thalassaemia). Heterozygosity for a rare polymorphism (HBB:c.315+26T>G, dbSNP rs368604295) not known to be of any clinical significance was also detected. GAP-PCR showed none of the most common types of alpha zero (--SEA,--FIL, --THAI,--MEDI, -(a)20.5kb or --BRIT) or alpha plus (-a3.7kb or –a4.2kb) thalassaemia or the anti3.7 triple alpha was present. Carrying beta thalassaemia is sufficient to explain the haemoglobin disorders' screen results. It is often associated with slight anaemia but other causes of anaemia have not been excluded by these tests and serum ferritin should be measured.	Information sheets and haemoglobinopathy cards are also provided and a Proforma issued for referral to a Genetic Counsellor. Note for 1401DN1: as this was the first rs368604295 had been seen in this laboratory it was investigated for the possible activation of a cryptic splice site by testing the altered sequence in 3 Splice Site programme (Netgene2, NNSplice and Human Splicing Finder). No difference from the control sequence was found. The Ensembl database, accessed 07.05.14, flagged no deleterious consequences and indicated no overlap with Ensembl regulatory features or motif features for this SNP.
	No results returned	

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APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	Sample identification: 1401DN2 Material: Analyses are performed on extracted DNA from UKNEQAS. Result: The patient is heterozygous for HBB:c.19G>A, and the mutation gives rise to Hb C. The patient is also heterozygous for HBB:c.20A>T, and this mutation gives rise to Hb S, (Reference: the HbVar database). The DNA-analyses performed cannot determine whether these two mutations are situated on the same allele (cis) or on different alleles (trans), but since the HPLC performed by UKNEQAS shows fractions consistent with both Hb C and Hb S the mutations must be placed on different alleles (trans). The patient is also heterozygous for the alpha 3.7 deletion (αα/-$\alpha^{3.7}$). The alpha 3.7 deletion causes alpha thalassemia and the patient expresses three instead of four alpha-globin alleles. Interpretation: The patient has Hb SC disease with co-existing heterozygosity for alpha thalassemia 3.7. The patient should be referred to a clinical haematologist due to the nature of this disease. These genetic traits can be transferred to future generations. Both Hb C and Hb S can in combination with certain other genetic haemoglobin mutations result in severe disease. The alpha thalassemia 3.7 is almost always associated with a mild haematological phenotype regardless heterozygous or homozygous form. Genetic counselling may be required later on in life.	Methods used:MLPA, HB alfa-locus (MRC Holland) MLPA, HB non alpha locus (MRC Holland) Sanger sequence HBB (NM_000518.4) Sanger sequence HBA1 (NM_000558.3) Sanger sequence HBA2 (NM_000517.4)

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APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	Results: PCR testing indicates a 3.7kb deletion of a single alpha globin gene. PCR testing for the triplication of alpha gene was normal. Beta Globin Sequencing NG_000007.3(HBB):c[19G>A, p.Glu7Lys] ; [20A>T, p. Glu7Val] DNA sequencing of the beta-globin gene has detected a heterozygous, single base change (A>T) that causes a change from Glutamic Acid to Valine (GAG>GTG) at amino acid 6 of the mature protein. This result is consistent with Haemoglobin S Trait. In addition, DNA sequencing of the beta-globin gene has detected a heterozygous, single base change (G>A) that causes a change from Glutamic Acid to lysine (GAG to AAG) in amino acid 6 (6.1) of the mature protein. This abnormal haemoglobin is known as Haemoglobin C. **Interpretive Comment:** Alpha globin genotype: $\alpha\alpha/\alpha^{-3.7}$ Results consistent with co-existent Haemoglobin S and Haemoglobin C traits and Alpha Thalassaemia trait. Compound heterozygosity for Haemoglobins S and C produces a phenotype of Sickle Cell disease. The patient should be referred to a specialist centre for further management. In addition, there is a reproductive risk of Haemglobin H disease if the alpha thalassaemia mutation is co-inherited with more extensive alpha gene deletions (i.e. those where both alpha genes on one chromosomes are deleted). Partner testing, family studies and genetic counselling should be considered where appropriate.	

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APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	HPLC indicated the presence of both Hb S and Hb C and sequencing revealed 1401DN2 was compound heterozygous for Hb S and Hb C. Alpha genotyping detected Alpha 3.7 deletion and is a carrier for Alpha + Thalassaemia. The parents of 1401DN2 should be checked for the presence of both Hb S and C, in addition to Beta Thalassemia indices.	
	Haemoglobin SC disease (Codon 6 GAG>GTG and Codon 6 GAG>AAG). These mutations are usually associated with a moderate to severe sickle cell disease. Patient is also a carrier for alpha^{+} thalasaemia ($-\alpha^{3.7}$ deletion). This patient should be referred to consultant Haematologist /Haemoglobinopathy clinic.	
	Hb SC disease and alpha plus thalassaemia trait. DNA studies show compound heterozygosity for the Hb S mutation (Cd6 GAG>GTG) [HBB:c.20A>T] and the Hb C mutation (Cd6 GAG-AAG) [HBB:c.19G>A], genotype SC, and heterozygosity for 3.7 kb single alpha globin gene deletion (genotype: -a3.7/aa). Hb SC disease is a sickling disorder of variable severity but usually has a more benign phenotype than HbSS disease.	

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APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	Multiplex GAP PCR amplification of alpha globin gene for alpha zero thalassaemia deletions (SEA, MED, FIL, THAI and 20.5) and for the alpha plus thalassaemia deletions (3.7 and 4.2), indicates the patient is heterozygous for the alpha plus 3.7 deletion (aa/a~3.7), i.e. alpha plus thalassaemia trait. DNA analysis by Sanger sequencing confirms the variant (HBB:c.19G>A) in Beta codon 6 GAG > AAG (Glu>Lys) in addition to the variant (HBB:c.20A>T) in Beta codon 6 GAG > GTG (Glu>Val). This data is consistent with compound heterozygosity for Hb C and Hb S. Technical limitations make it impossible to confirm whether these mutations are in cis or trans; please interpret together with the haematological results.	
	Genotyping for the main deletions of the alpha globin loci reveal a heterozygous 3.7kb deletion ($-\alpha^{3.7}/\alpha\alpha$). This patient has a co-existing haemoglobinopathy and α^{+} thalassaemia. Beta globin gene analysis was not performed.	
	The molecular test confirms thè haematological phenotype: thè patient is heterozygous for alpha thalassemia type 3.7 ($-\alpha^{3.7}/\alpha\alpha$) and compound heterozygous for HbS/HbC ($\beta^{Cd6(A>T)}/\beta^{Cd6(G>A)}$). The copresence of β^{S} and β^{C} leads to a sickling disorder similar to sickle cell anemia (SCA) but less severe. The coexistence with alpha thalassemia type 3.7 ($-\alpha^{3.7}/\alpha\alpha$) generally helps to make less severe thè clinical picture due to thè β defects. Parental testing, genetic counselling and patient follow up are suggested. In order to assess thè degree of clinical severity it should be also evaluated thè percentage of thè different hemoglobines by HPLC, in 3 or 4 months.	

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APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	The patient is heterozygous for an α^{+} allele ($-\alpha^{3.7}$). With the techniques used, we can not exclude the possibility of rare deletions or mutations. Taking into account these limitations, the genotype found is compatible with the diagnosis of a silent alpha-thalassemia. It is most often asymptomatic or associated with microcytosis. At minimum, a blood count and an haemoglobin screen should be done on parents. Alpha-thalassemia mutation screening should also be proposed to parents to exclude the risk of Hemoglobin H disease in future pregnancies	1401DN2 is a compound heterozygote for HbS and HbC mutations which correspond to a major sickling disorder. It may be symptomatic after the haemoglobin switch will be complete because of HbF expression reduction. However, HbS and HbC expression may be slightly reduced owing the co-occurrence of a 3.7 alpha-thalassemia deletion. Genetic counselling should be offered to the parents
	Hb SC disease with co-inheritance of α–thalassaemia β^{S}/β^{C} **HGVS:** NG_000007.3:[g.70614A>T] ; [70613A>T] $-\alpha^{3.7}/\alpha\alpha$ **HGVS:** NG_000006.1:g.34164_37967del3804 Appointment with pediatric hematologists should be offered. Parent education program towards HbSC disease should be offered.	
	3 month old female infant with HbSC disease. A family work up is recommended.	

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APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	DNA investigation of the α-gene and β-gene locus revealed a different base substitution in both β-globin genes. The $\beta^{cd6(A>T)}$ substitution confirmed the presence of HbS and the $\beta^{cd6(G>A)}$ substitution confirmed expression of HbC. One of the 4 α-genes is missing. The switch from foetal haemoglobin expression to expression of the adult form will be complete between the age of 6 months and a year and result in expression of almost solely HbS and HbC. Patients with this combination, HbSC, suffer from sickle cell disease. The co-existing mild form of α-thalassaemia might reduce clinical severity to some extent. We strongly suggest consultancy of a clinic specialized in treatment of sickle cell disease at short notice, at least before the age of 6 months. Genetic counselling is recommended, including the partner of the mother and first degree relatives of the patient.	
	This African infant with mild anaemia and abnormal fraction of Hb electrophoresis is compound Ht for HbC HbS. She is also α thal. Silent carrier. Genetic counselling should be provided to the family.	
	Is a heterozygous for α^+ thalassaemia due to the 3.7 Kb deletion. Hematological results are consistent with a compound heterozygous Hb S/ Hb C in association with heterozygosity for alpha plus thalassaemia. Genetic counselling should be offered.	Beta genotype is performed in laboratory participant reference number 29510.

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	Haematological interpretation	Additional comment
	The patient is heterozygous for two mutations at codon 6 level of the β-globin gene: the G>A mutation, specific of HbC and the A>T mutation, specific of HbS (HBB{NG_000007.3}:g;70613G>A and g.70614A>T). The genotype is β^S/β^C compatible with SC disease. The results are consistent with those of blood analysis. The patient is also heterozygous for the alpha 3.7 deletion (genotype – $\alpha^{3.7}//\alpha\alpha$). The sequence of the α1 and α2 genes is normal. Genetic and referral to a Consultant Haematologist is recommended.	
	No comments given.	
	The result shows the HbS and HbC anomalia in the ß-globin gene in the combined heterozygous state. Furthermore, the –α-3.7 deletion in the α-globin gene complex was detected in the heterozygous state. The detected genotype leads to the HbSC disease in combination with a heterozygous α(+)-thalassemia. The resulting phenotype is mainly characterized by a slight change in the hematologic profile. Patients usually show a mild phenotype with a mild hemolytic anemia. Nonetheless, patients can experience symptoms similar to homozygous sickle cell anemia. The –α3.7 deletion does not contribute to the phenotype of the patient. Offspring inherits each of the mutations with a risk of 50%. According to the detected genotype the parents of the patient are at least heterozygous carriers of the detected mutations. We recommend genetic counseling. No mutations in the α-globin genes HBA1 and HBA2 were detected. The MLPA analysis showed no deletion in the ß-globin gene complex.	

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APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	Sickle cell disorder due to co-inheritance of Hb S and Hb C. Heterozygous for the alpha 3.7 deletion. Close relatives (parents, siblings) should be sent for hematological evaluation.	
	Hb SC. Hb SC disease. Alpha genes not tested. HbF and HbA2 according to age.	
	β-gene analysis: The sample was sequenced and found compound heterozygote for HbS (cd6 GAG>GTG) and HbC (GAG>AAG) Genotype: $\beta^{HbS(cd6A>T)}/\beta^{AHbC(cd6G>A)}$ α-gene analysis: The sample was also analysed by MLPA (α-locus) and α2 and α1 genes were sequenced. The sample was found to be heterozygote for $-\alpha^{3.7}$. Genotype: $\alpha\alpha/-\alpha^{3.7}$	High HbF in 1402DN2 is due to the age of the infant being less than one year old.

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APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	The results obtained revealed that **1401DN2 patient is heterozygous for the c.19G>A and c.20A>T mutations, both present in exon 1 of the *HBB* gene.** The mutation c.19G>A changes codon 6 coding for Glutamic acid into Lysine (p.Glu6Lys) giving rise to Hb C variant. The mutation c.20A>T changes codon 6 coding for Glutamic acid into Valine (p.Glu6Val) giving rise to Hb S variant. This result confirms the absence of Hb A in the patient. **The patient should be a compound heterozygous for the sickle cell and the HbC mutations. 1401DN2 genotype is consistent with his haematological and biochemical data (see also alfa-globin genotype).** Close relatives of 1401DN2 patient (parents, siblings) should be sent for genetic counselling and a cascade molecular analysis is recommended in this family. After confirming the parental mutations, and in case of a new pregnancy, pre-natal diagnosis can be offered to the parents of 1401DN2 patient. Haematological evaluation and genetic counselling is also recommended to other close relatives of this family.	
	The patient has sickle cell disease caused by compound heterozygosity for HbC and HbS. In addition, a heterozygous -alpha3.7(Rightward) deletion was detected, the patient is therefore also carrier of a mild alpha-thalassemia (type -,alpha/alpha,alpha). Genetic counselling for parents of consultand and family members is indicated, carreir testing of the future partner and first degree relatives is recommended.	

APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	Hemoglobin SC disease plus mild α^+ thalassemia. Genotype: HBB:c.19G>A / HBB:c.20A>T plus $-\alpha^{3.7}/\alpha\alpha$ The Hemoglobin SC disease is characterized by a much milder anaemia and fewer crises than sickle-cell anaemia. Genetic counselling and family testing is indicated.	
	Alpha Globin Gene (using MLPA): Heterozygous $-\alpha 3.7$ deletion found. **Alpha Globin Gene (using GAP-PCR**): *Positive* for the $-\alpha$3.7 heterozygous deletion mutation. **Beta Globin Gene (using fluorescent DNA sequencing):** Positive for the heterozygous codon 6 (GAG>GTG) HbS mutation [HBB:c.20A>T] Positive for the heterozygous codon 6 (GAG>AAG) HbC mutation [HBB:c.19G>A] **Conclusion:** Result for this baby is HbSC disease with coincidental alpha-plus thalassaemia trait. The presence of the alpha-plus thalassaemia trait may have an effect to reduce the severity of the phenotype of HbSC disease. HbF for this baby is 51% which consistent with the age of the baby. **Please confirm results at 6 months of age.** **Baby must be referred to the local Sickle Cell and ThalassaemiaSpecialist Clinic immediately as the national recommendation require children affected with sickle cell disorders to be seen in specialist clinics by 3 months of age.**	

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APPENDIX 2: Interpretive comments for 1401DN2

	Haematological interpretation	Additional comment
	The patient is a compound heterozygous carrier of the two mutations c.19G>A and c.20A>T in codon 6 of the HBB gene, leading to the two haemoglobin variants HbC and HbS, respectively. These findings confirm the abnormal hematological values and the HPLC analysis. The additionally found heterozygous -3.7 deletion of the HBA2 gene may have a moderating impact on the disease. The high HbF value is referable to the young age of the patient. Genetic counselling should be offered to the parents and cascade screening of family members is recommended.	
	Patient is a compound heterozygote for the beta S and Beta C mutations. This genotype confirms the hemoglobin study and corresponds to a phenotype of sickle cell syndrome (patient SC). A heterozygous alpha -3.7 deletion was also identified as part of the systematic screening of modifier genes. Clinical follow-up by a specialized paediatrician is recommended. Parents of the child should be addressed to genetic counselling.	

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	Haematological interpretation	Additional comment
	Sequence analysis of a sample of genomic DNA from this individual has identified her as apparently compound heterozygous for the c.19G>A, p.(Glu7Lys) and c.20A>T, p.(Glu7Val) missense mutations in exon 1 of the *HBB* gene. These common mutations are referred to as haemoglobin C and haemoglobin S, respectively. **These results are consistent with a diagnosis of haemoglobin SC disease in this individual.** Further analysis using gap PCR to detect large deletions of the HBA genes has shown that this individual is also heterozygous for the $-\alpha^{3.7}$ deletion of the *HBA1* and *HBA2* genes. She lacks the deletion mutations $^{--MED, --FIL, --SEA}$ and $-(\alpha)^{20.5}$. Other haemoglobin mutations, including non-deletion mutations have not been investigated. **This individual is thus also a carrier of alpha thalassaemia.** Together, these results are consistent with the red cell indices in this individual. We recommend referral to the Clinical Genetics service where the implications of these findings can be discussed. Cascade screening of other family members, who are at an increased risk of being carriers and thus of having a child affected with Beta-Thalassaemia, can also be discussed. This report is subject to the pedigree being as stated. DNA has been stored from this individual. If you require any further information on these results, please do not hesitate to contact the undersigned.	
	This baby is a compound heterozygote for Sickle Cell and Hemoglobin C (HBB:c.20A>T; HBB:c.19G>A). The high percentage of fetal hemoglobin is typical in first months of age but it will decrease with time. This baby also carries a single alpha-globin gene deletion (NG_000006.1:g.34164_37967del3804) which probably does not have a pronounced impact on the clinical presentation. This condition is variable in its phenotypic manifestations and therefore the child will require a life long follow by physicians. The parents should be recommended to consider prenatal diagnosis in future pregnancies.	

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	Patient is compound heterozygous for HbS and HbC. Additionally, the HBA2/ HBA1 -3.7 single gene deletion could be detected that phenotypically corresponds to an heterozygous α^{+} thalassaemia	
	Result: • Beta globin genotyping: Compound heterozygous genotype: c.[19G>A];[20A>T] Methods: PCR amplification of the beta globin gene (NM_000518.4) and sequencing promoteur, exonx, introns and polyA sequence. • Alpha globin genotyping: Heterozygous for alpha thalassaemia type 3.7. Apha genotype: -α3.7/$\alpha\alpha$ Methods: Gap PCR for –α3.7 and $\alpha\alpha\alpha$anti3.7 Interpretation The patient has sicle cell disease: haemoglobin SC disease (β^{S}/β^{C}) and is also heterozygous for alpha-plus thalassaemia trait type 3.7 (-α3.7/$\alpha\alpha$). The haemoglobinopathy requires specific medical care.	
	No results returned	
	Patient is affected by a SC Sickle Cell Syndrome. The family should be referred to a specialised center for appropriate care. In addition, she is carrier of several variants known to modify the time course of the disease.- alpha + thal trait ($\alpha^{-3.7}$ heterozygous) - benin/benin beta globin cluster haplotype (low F) - Gilbert syndrome	
	No comments given	

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	Haematological interpretation	Additional comment
	The sequencing of the β globin genes confirms the following genotype : β^S / β^C. Like sickle cell anemia, the haematological and clinical features of HbSC disease are heterogeneous, but all of the complications that make sickle cell anemia notorious can be present. Proliferative sickle retinopathy is perhaps the most typical vasoocclusive complication of HbSC disease. The patient should register for an ophthalmological follow up. The patient is also heterozygous for the $-\alpha^{3.7}$ deletion.	
	No results returned	
	Sample compound (double) heterozygous for hemoglobin S (HbS) and hemoglobin C (HbC) HbS/HbC. No analysis for a-thalassaemia	

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	Haematological interpretation	Additional comment
	DNA sequence analysis has shown that **1401DN2** is compound heterozygous for the beta globin variants Hb S and Hb C. This is consistent with a diagnosis of Haemoglobin SC disease, which can result in a sickling disorder that is associated with chronic haemolytic anaemia. **1401DN2** is also heterozygous for the 3.7 kb single alpha-gene deletion (alpha plus thalassaemia trait) which has no additional clinical significance. If not already undertaken, **1401DN2** should be referred for appropriate monitoring of her clinical condition. Screening of any future partner of **1401DN2** is indicated to establish if there is a risk to any future offspring for inheritance of a clinically significant haemoglobinopathy. If not already undertaken, screening of **1401DN2**'s parents is indicated as there is a 1 in 4 risk for any of their offspring to inherit Hb SC disease, assuming no further mutations are present in either individual. **Conclusion:** **1401DN2 has haemoglobin SC disease. We recommend testing of any future partner to determine the risk of having offspring with a clinically significant haemoglobinopathy.**	The Hb F level present in 1401DN2 is consistent with her age; therefore no further investigation would be performed.
	INDICATION:Suspicion of a sickle cell disorder.METHOD:PCR amplification followed by direct sequencing of HBB gene.Our reference sequence is the coding sequence NM_000518.4 (A from ATG =1)RESULT:Identification of the p.Glu6Lys and p.Glu6Val mutation in the heterozygous state.Genotype: c.[19G>A]; [20A>T]CONCLUSION:Molecular confirmation of a sickle disorder, a compound heterozygosity for HbS and HbC.A genetic counselling is recommended.	
	No comments given	
	Result: Mutations HBB: c.20A>T, p.Glu7Val (E6V) and HBB:c.19G>A, p.Glu7Lys(E6K) identified at the heterozygous composite state. Conclusion: this analysis confirms a diagnosis of sickle-hemoglobin disease	We don't use the β^A/β^A nomenclature in our reports. We use the HGVS nomenclature in our report and the accession number of the

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	Haematological interpretation	Additional comment
	(HbSC) Phenotype: Milder hemolysis and anemia. A familial study is recommended. A genetic consultation and parent testing are recommended.	gene is given in our report in the " technical session/".We also keep the "ancient" nomenclature like E6V for sickle cell disease mutation in our report.
	Haemoglobin SC disease. Heterozygous alpha-plus-Thalassemia type -3.7. Comment: HbF increase due to the age of the patient.	
	The supplied laboratory results prompted us to check for the presence of beta-globin variants. The beta-strip (ViennaLabs) revealed compound heterozygosity for the mutations HbS (*HBB:c.20A>T*) and HbC (*HBB:c.19G>A*), which is in accordance with the HPLC results. We additionally checked for common deletions in the HBA gene cluster using gap-PCR (deletions tested for: 3.7, SEA, MED, 20.5, FIL, and THAI). The alpha-3.7-deletion was detected in a heterozygous state. These results are consistent with HbSC disease. The condition is alleviated by the presence of the alpha-3.7-deletion (Powars et al., Am J Hematol. 2002 Jul;70(3):206-15). We recommend family testing. These results should be communicated in the context of a genetic counselling.	We don't use the $\alpha\alpha/\alpha\alpha$ and β^A/β^A nomenclature in our reports. Poorly designed result sheet.

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	NEQAS 1401DN2 is compound heterozygous for Hb S, codon 6 (GAG;Glu > GTG;Val) and Hb C, codon 6 (GAG;Glu > AAG;Lys) mutations in the beta globin gene. This is a sickle cell disease genotype. Suggest referral to a haematologist at a local sickle cell clinic. The sample is also heterozygous for alpha + thalassaemia due to the 3.7 kb deletion. One of the four alpha globin genes has been deleted from one chromosome. These genetic results are consistent with the phenotypic findings for a child of this age. DNA used in this diagnosis has been stored indefinitely. If you have any queries concerning this result, please do not hesitate to contact the laboratory.	

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	Haematological interpretation	Additional comment
	THIS IS AN UPDATED AND FINAL REPORT SICKLE CELL DISORDER, COMPOUND HETEROZYGOUS HbS/HbC TYPE (BETA6 GLU>VAL/BETA6 GLU>LYS) also known as HBB:c.[20A>T]+[19G>A] ALPHA GLOBIN GENOTYPE IS –a3.7kb/aa THIS RESULT INDICATES THE PATIENT HAS A SERIOUS INHERITED HAEMOGLOBIN DISORDER AND SHOULD BE REFERRED TO A PAEDIATRIC HAEMATOLOGY CONSULTANT FOR CLINICAL EVALUATION. This has implications for other family members for which INFORMATION AND GENETIC COUNSELLING MUST BE PROVIDED. Full blood count and haemoglobin disorders' screen indicated an infant with borderline low indices and fractions in the HbS, HbC and HbF windows on HPLC. The %HbF was as expected for 3months of age. A small fraction in the HbA2 position was also noted. Referred for DNA studies to confirm the nature of the haemoglobinopathy present. Beta globin gene sequence analysis confirmed compound heterozygosity for Hb S and HbC. The fraction in the A2 position would be that expected of a HbS derivative so no further investigation of that was carried out. GAP-PCR for alpha thalassaemia showed heterozygous -a3.7kb alpha plus thalassaemia. None of the common forms of alpha zero (--SEA,--FIL,--THAI,--MEDI, -(a)20.5kb or --BRIT) nor the –a4.2kb form of alpha plus thalassaemia nor the anti3.7 triple alpha was found.	Information sheets and haemoglobinopathy cards are also provided and a Proforma issued for referral to a Genetic Counsellor.
	No results returned	

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