Oxford University Hospitals NHS
NHS Foundation Trust

NHS

Screening Programmes

Sickle Cell and Thalassaemia

The Sickle Cell and Thalassaemia Helpline – what have we learnt?

UK NEQAS Haematology 21st Annual Participants Meeting
October 2018

UKAS
UNITED KINGDOM ACCREDITATION SERVICE

Oxford Molecular Haematology Diagnostic Service

UNIVERSITY OF OXFORD

NHS
National Institute for Health Research

Goals of Lab Support Service

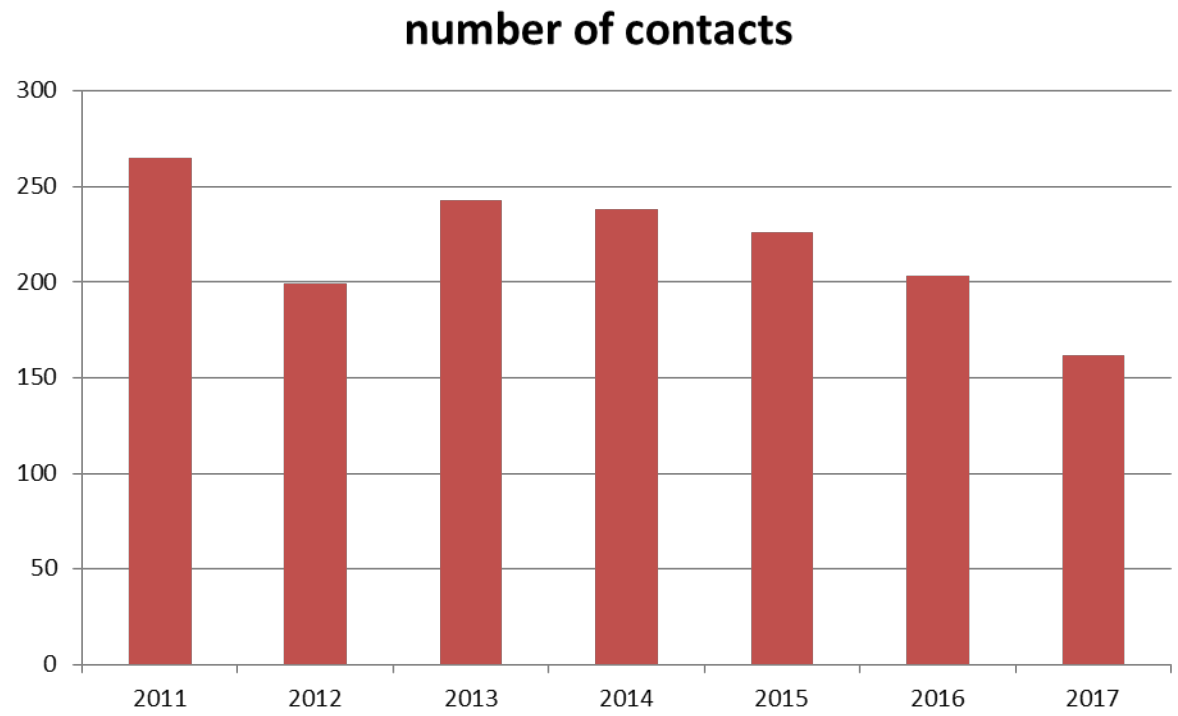
- To provide support for sickle cell and thalassaemia screening laboratories (telephone and e-mail – secure nhs.net account)

- Review of the type of queries the service is receiving will the allow the national programme to monitor topics that are causing concern at local level and may require review nationally.

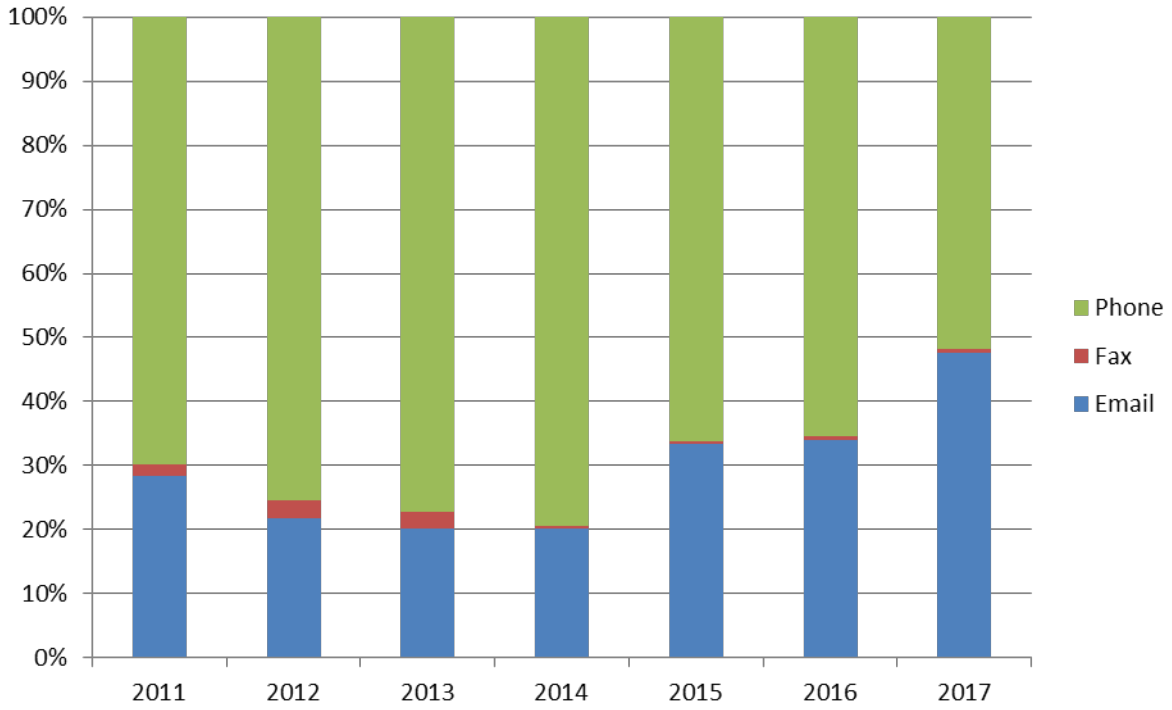
2010:- Following a competitive tendering exercise,
The National Sickle Cell and Thalassaemia Screening Programme commissioned the Oxford National Haemoglobinopathy Reference Laboratory (NHRL) to provide a support service for screening laboratories.

- Oxford provides antenatal screening, newborn screening and genetic testing for haemoglobinopathies including prenatal diagnosis.
- Experience of the three arms of the screening programme linking up and working as a whole.
- Able to offer advice and support which is based on extensive experience in all these areas.

Laboratory support service calls – complete years (2011-2017) (total ~ 1650)
From >200 different locations within the UK

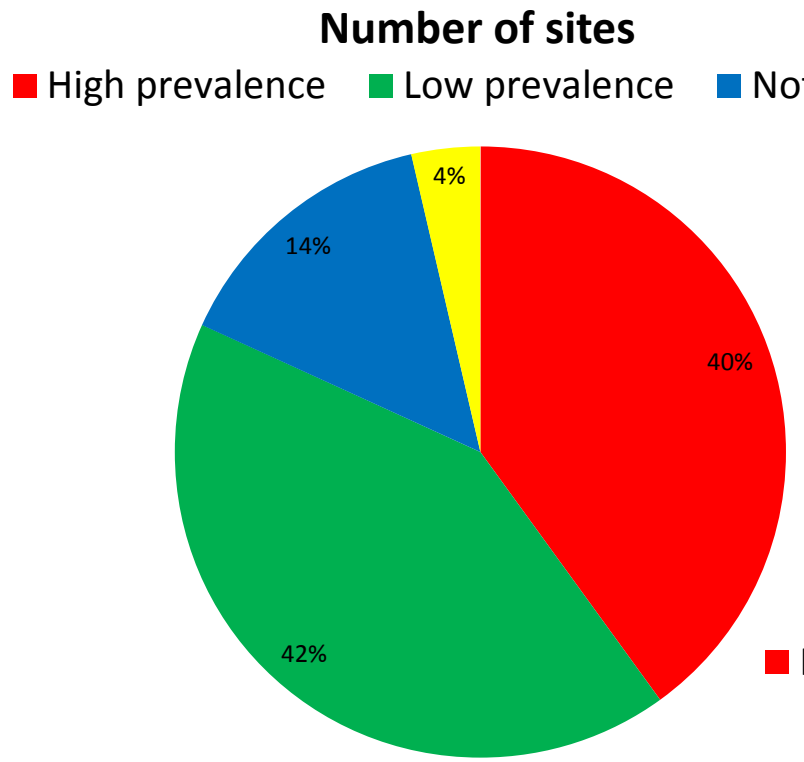


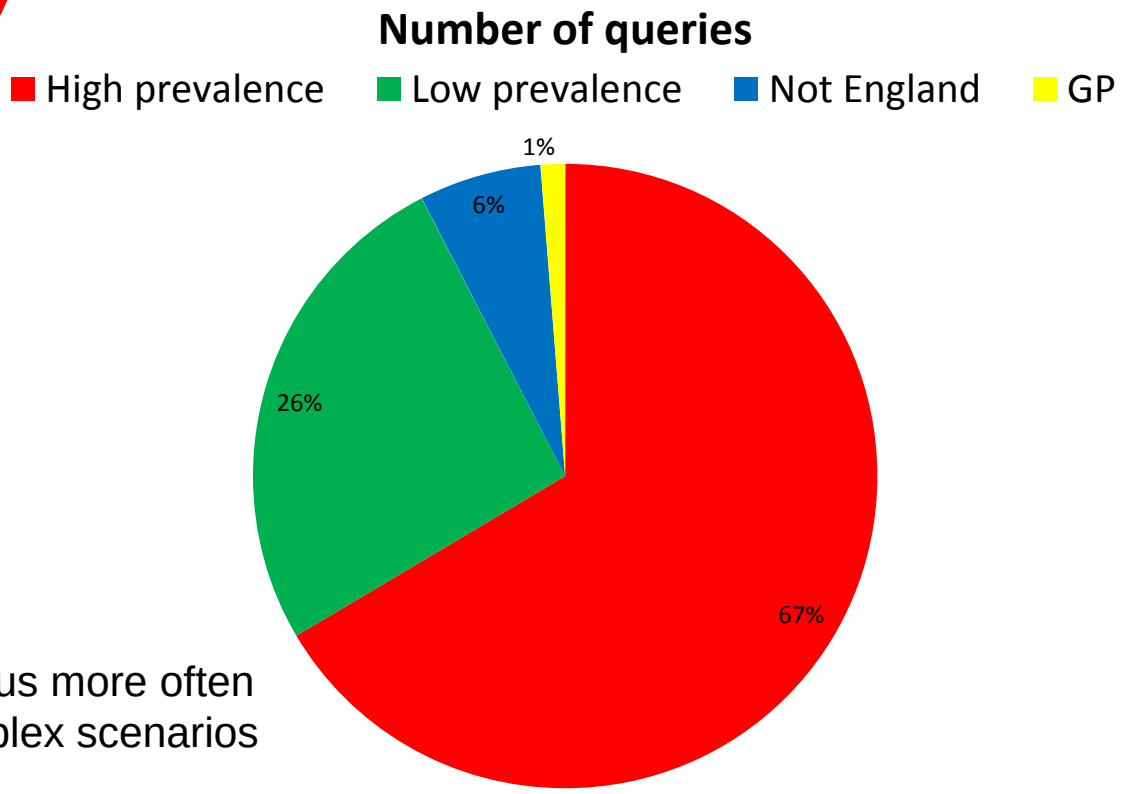
Modes of contact



Email is becoming more common

Fax/letter is consistently rare – faxes not permitted at OUH Trust

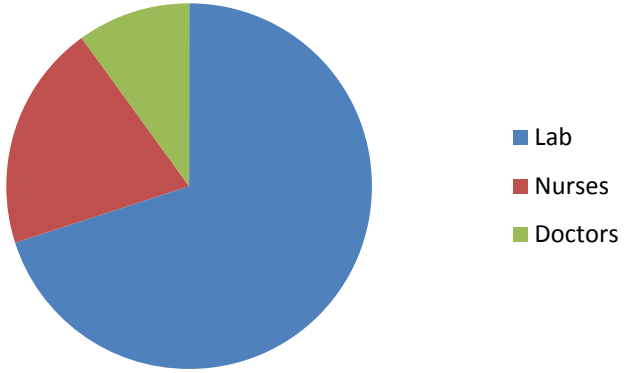


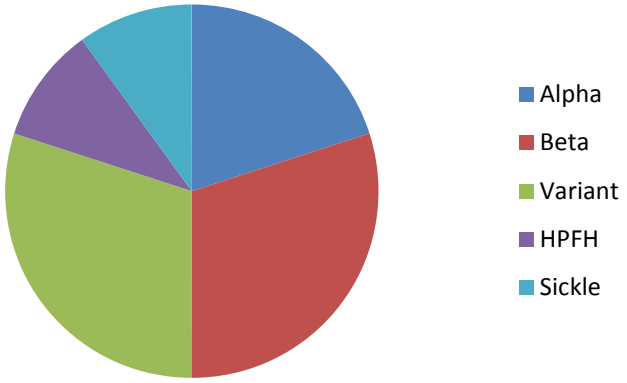


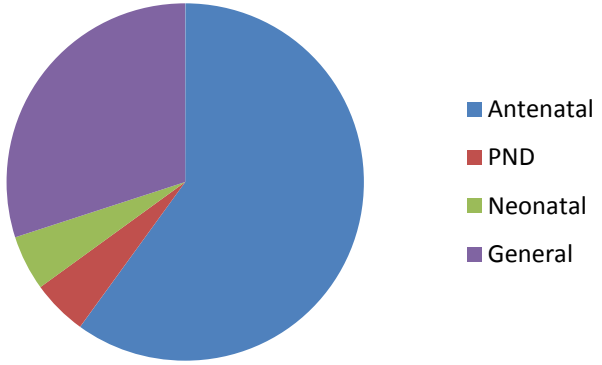
High prevalence sites tend to contact us more often – they encounter many different complex scenarios

Types of enquiries

- Enquirers:
  - 70% from laboratory staff
  - 20% from nurses/midwives/counsellors
  - 10% from clinicians
- Type of enquiry:
  - Largest proportion involve antenatal screening
  - Followed by “routine hbopathy”
    - eg diagnostic cases/incidental findings
  - Newborn screening and PND enquiries are relatively rare
- Subject of enquiry:-
  - Variant and ?beta thalassaemia enquiries are currently the most common
  - They have overtaken alpha thalassaemia enquiries which were very frequent before clear guidance was established in the screening handbook







Common themes have changed over time

- Queries that were **very** common now less so:-
  - Is “x” classified as a high risk area for alpha zero thalassaemia
  - How to identify a rare haemoglobin variant
  - Borderline A2 levels

- Queries that have been consistently common:-
  - Significance of small peaks
  - Low A2/split A2/concerns about accurate quantitation
  - Significance of raised Hb F

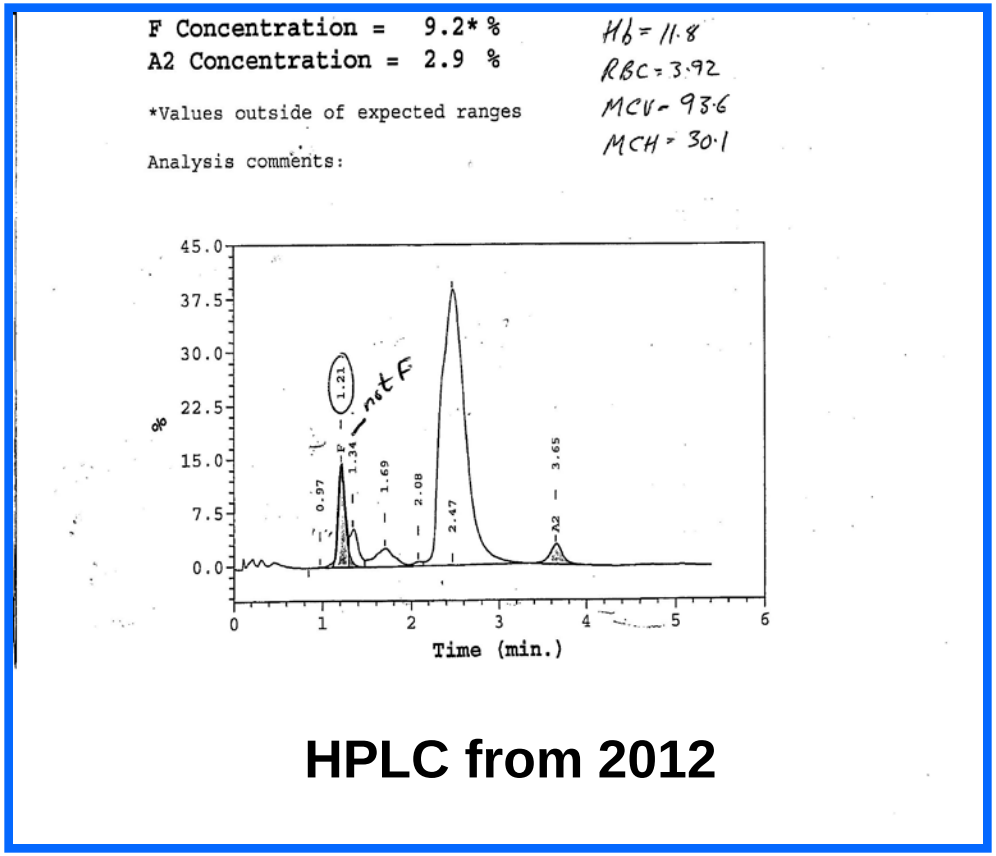
- Queries related to relatively new scenarios:-
  - Sperm donor/egg donor reproductive scenarios
  - Retesting in subsequent pregnancies resulting in altered diagnosis

Examples – repeat testing

- Pregnancy in 2007
  - MCH 26.4 HbA2 3.5
  - Reported as a beta thalassaemia carrier
  - Partner testing initiated (normal)
- Pregnancy in 2013
  - MCH 26.1 HbA2 3.2
  - Reported as a possible alpha plus carrier
- Pregnancy in 2015
  - MCH 27.5 HbA2 3.2
  - Reported as normal
- Under current guidelines the original diagnosis would have been “possible” beta thalassaemia carrier

Examples – repeat testing

- Pregnancy in 2009
  - HbA2=3.2 F=0.2, HbA=80.4, P2 Peak 7%
  - Hb, MCV, MCH - normal
  - Called ‘Normal’ – looked like a diabetic HPLC
- Pregnancy in 2012
  - HbA2=2.9 F=9.2, HbA=79.1, P2 =3.6
  - Difference in HbF ? – IEF performed – variant found



HPLC from 2012

Examples – reproductive scenarios

- **Ideally start with testing at least one accessible biological parent!**
  - (also test pregnant lady – even if there are no reproductive risks she may have a clinically significant haemoglobinopathy that could become apparent during pregnancy)
- E.g. same sex couple (female), one partner is surrogate, other is egg donor (sperm provided via fertility clinic)
- E.g. donor egg twin pregnancy, no info on egg donor, father is a sickle carrier
- **If neither biological parent is available or normal, alert screening coordinator to investigate results via fertility clinic**

Examples – variant haemoglobins

- Unless a variant has been previously characterised as benign in an earlier pregnancy (and the current results are consistent), partner testing is always required

- Even if you have a high suspicion of the nature of the variant, unless you have run appropriate controls alongside (as for Hb S, C, D, E) you cannot provide a clear diagnosis without confirmation by DNA or mass spectrometry

- Usually it is quicker and cheaper to screen the partner

Hb G-Philadelphia
+ alpha plus thalassaemia (hom)
Benign alpha variant mimicking beta variant %

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.4	---	1.09	7151
Unknown	---	0.6	1.26	9524
P2	---	2.4	1.36	40916
P3	---	2.6	1.74	43707
Ao	---	49.3	2.46	838863
A2	0.8*	---	3.62	13467
D-window	---	42.9	4.10	729268
S-window	---	1.0	4.49	17400

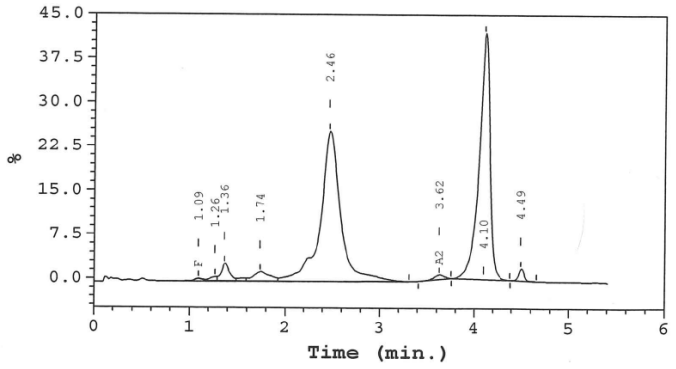
Total Area: 1,700,295

F Concentration = 0.4 %
A2 Concentration = 0.8* %

*Values outside of expected ranges

Analysis comments:

Hb G-Phil
+
$-\alpha^{3.7}/-\alpha^{3.7}$



Hb Hammersmith
Haemolytic anaemia,
MCH normal, A2 borderline,
Hb F variably raised

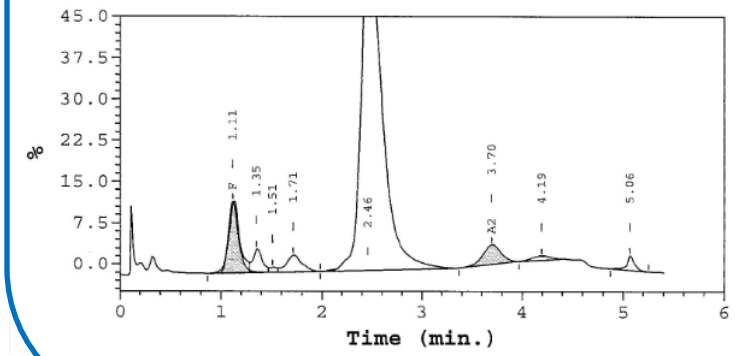
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	8.3*	---	1.11	170746
P2	---	2.5	1.35	51569
Unknown	---	0.4	1.51	9068
P3	---	2.9	1.71	61046
Ao	---	79.8	2.46	1669841
A2	3.6*	---	3.70	85071
D-window	---	0.9	4.19	18278
C-window	---	1.3	5.06	26856

Total Area: 2092475

F Concentration = 8.3* %
A2 Concentration = 3.6* %

*Values outside of expected ranges

Analysis comments:



Increased levels of Hb F + hypochromia

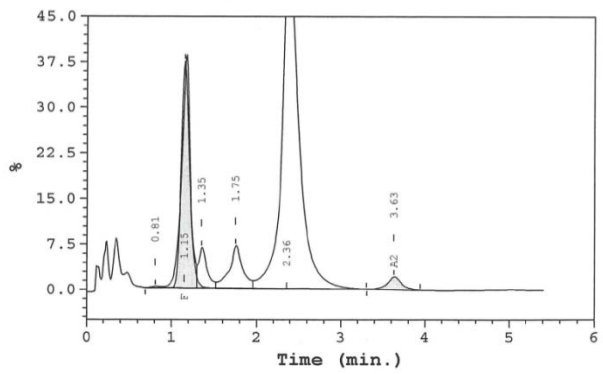
Alpha thalassaemia + HPFH

Hb	165
RBC	6.77
MCV	77
MCH	24.4

F Concentration = 21.9* %
A2 Concentration = 2.2* %

*Values outside of expected ranges

Analysis comments:



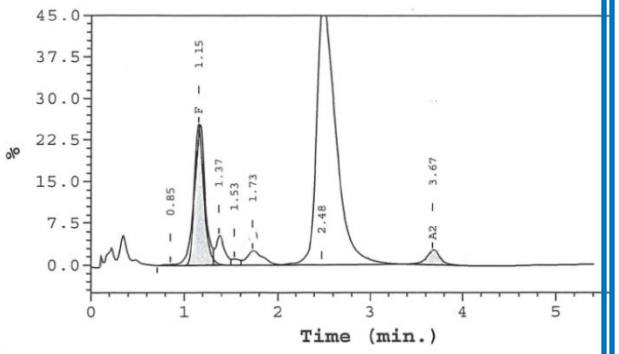
Delta-beta thalassaemia

Hb	112
RBC	4.57
MCV	76
MCH	24.5

F Concentration = 19.8* %
A2 Concentration = 2.7 %

*Values outside of expected ranges

Analysis comments:



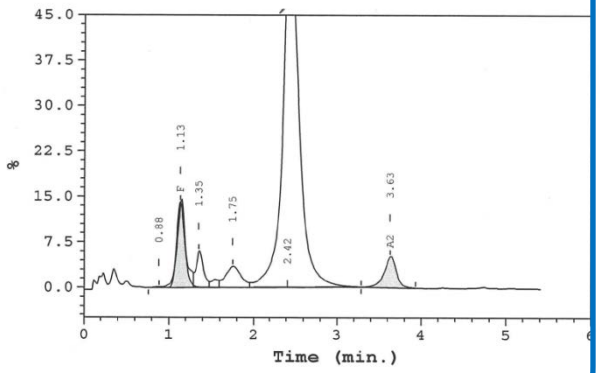
Beta thalassaemia + ndHPFH

Hb	122
RBC	6.64
MCV	57
MCH	18.4

F Concentration = 9.2* %
A2 Concentration = 5.2* %

*Values outside of expected ranges

Analysis comments:



Variable appearance of alpha chain variant on HPLC

G-Phil on it's own

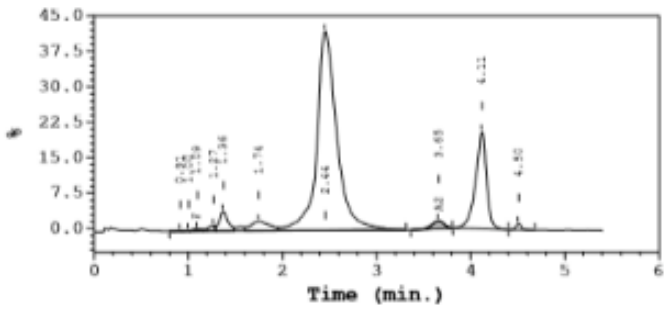
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
P1	---	0.0	0.91	612
Unknown	---	0.1	1.00	1174
F	0.3	---	1.09	7531
Unknown	---	0.7	1.27	16473
P2	---	2.9	1.36	65324
P3	---	3.2	1.74	70192
Ao	---	71.4	2.44	1587939
A2	1.6*	---	3.65	37913
D-window	---	19.1	4.11	425495
S-window	---	0.5	4.50	11687

Total Area: 2,224,339

F Concentration = 0.3 %
A2 Concentration = 1.6* %

*Values outside of expected ranges

Analysis comments:



G-Phil with homo 3.7

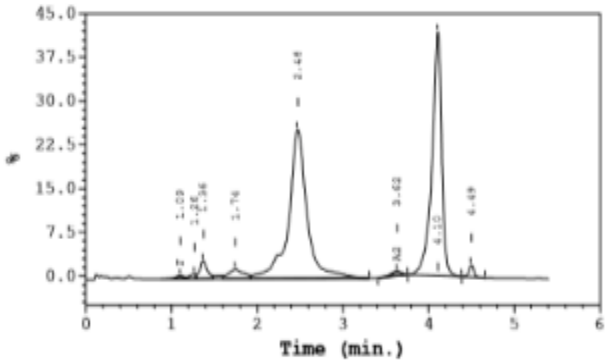
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.4	---	1.09	7151
Unknown	---	0.6	1.26	9524
P2	---	2.4	1.36	40916
P3	---	2.6	1.74	43707
Ao	---	49.3	2.46	838863
A2	0.8*	---	3.62	13467
D-window	---	42.9	4.10	729268
S-window	---	1.0	4.49	17400

Total Area: 1,700,295

F Concentration = 0.4 %
A2 Concentration = 0.8* %

*Values outside of expected ranges

Analysis comments:



Further examples

AS with G-Phil and alpha + carrier

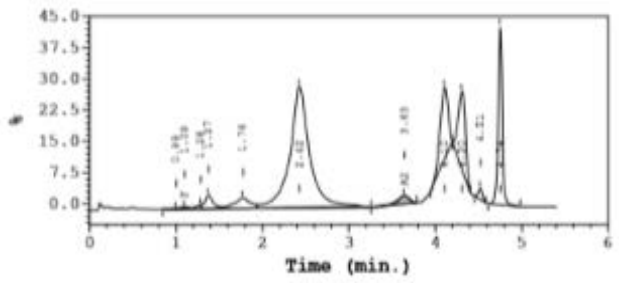
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.0	0.99	669
F	0.3	---	1.09	5445
Unknown	---	0.6	1.28	8842
P2	---	2.4	1.37	38874
P3	---	3.6	1.76	58248
Ao	---	51.1	2.42	818039
A2	1.9*	---	3.63	30813
D-window	---	12.7	4.10	202600
S-window	---	11.4	4.30	182915
Unknown	---	1.0	4.51	15368
Unknown	---	15.0	4.74	239583

Total Area: 1,601,395

F Concentration = 0.3 %
A2 Concentration = 1.9* %

*Values outside of expected ranges

Analysis comments:



SCD with G-Phil and alpha +

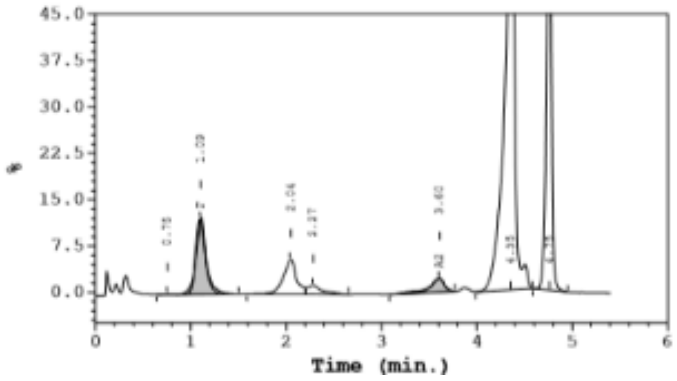
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
P1	---	0.1	0.75	1519
F	9.6*	---	1.09	161918
Ao	---	5.6	2.04	102880
Unknown	---	1.5	2.27	28304
A2	2.2*	---	3.60	45009
S-window	---	52.5	4.35	966092
Unknown	---	29.0	4.75	532797

Total Area: 1,838,520

F Concentration = 9.6* %
A2 Concentration = 2.2* %

*Values outside of expected ranges

Analysis comments:



User Survey June 2014
Results presented at last sub-group meeting (22/09/14)

Feedback Question	Maximum Points Available	Points Received	% Points received
How well is the service publicised?	200	125	62.5
Ease of access to the service	196	172	87.8
Response Time	192	176	91.7
Quality of the advice	192	183	95.3
Helpfulness of staff	192	184	95.8
Overall impression of the service	192	178	92.7

Please outline any way we might improve our service? (Quest feedback 2014)

- A member of staff could attend the Laboratory Updates run by the Screening Programme to publicise the service and put a face to the service.
- The helpline number should be on the front of the handbook/website and should be called ANNB Sickle/Thalassaemia helpline or Screening Programme Helpline
- More Publicity of the service.
- I have no complaints about the service, always responsive and helpful. Maybe a new publicising event to remind people.
- We have used this service on several occasions but I have found that I have had to tell colleagues about the service as they were unaware of the support that was available. Great service, just maybe needs publicising a little.
- I would like to know what type of queries come up across the board and then would be able to benefit and learn from the advice provided to others and see if their issues are also reflected locally.
- It would be useful to have a list of frequently asked questions available.
- Publicise it better. ?dedicated website.
- Expand online resources.
- An automated reply to email queries so we know they have been received would be reassuring
- This service is not widely advertised. ?website/internet etc. /or via BSH.

NSC UK National Screening Committee

NHS

Screening Programmes

Sickle Cell and Thalassaemia

Sickle cell and thalassaemia screening programme laboratory support service

Frequently Asked Questions

Alpha Thalassaemia

Q1: How do I find out if a particular country is high risk for alpha zero thalassaemia?
A: The following areas of the world are considered high risk for alpha zero thalassaemia (China, including Hong Kong, Taiwan, Thailand, Cambodia, Laos, Vietnam, Burma, Malaysia, Singapore, Indonesia, Philippines, Cyprus, Greece, Sardinia, Turkey, or if family origins are unknown). For screening purposes all other areas of the world are classified as low risk (Lab Handbook, 3rd edition, notes below testing algorithms). These areas are identified with a red hash on the family origin questionnaire.

Q2: I have a couple who are both from an area at high risk of alpha zero thalassaemia. One has an MCH below 25 and therefore possibly has alpha zero thalassaemia, the other partner has an MCH between 25 and 27. Do I need to carry out DNA testing to exclude the risk of Hb H disease?
A: No, testing for the risk of Hb H disease is not included in the sickle cell and thalassaemia antenatal screening programme. However, if fetal anaemia/hydrops is seen on ultrasound scanning or if there is a family history of hydrops fetalis, further investigations may be needed to exclude the possibility of the very rare condition of Hb H hydrops fetalis.

Q3: What do I do about alpha thalassaemia if one parent is from an area which is high risk for alpha zero thalassaemia and the other isn't?
A: For screening purposes alpha zero thalassaemia only needs to be considered if both parents are from high risk areas.

Q4: When do I need to consider masked alpha zero thalassaemia?
A: The presence of the carrier state for alpha zero thalassaemia can be masked by other co-existing haemoglobinopathies such as beta thalassaemia or Hb E. Therefore alpha zero thalassaemia must be considered whenever both parents' family origins are from high risk areas, regardless of any other haemoglobinopathy identified.

Q5: I have a couple from a high alpha zero thalassaemia risk area who both have an MCH below 25 and therefore might have alpha zero thalassaemia. What do I do next?

A: Alpha zero thalassaemia can only be definitively diagnosed by DNA testing, therefore parental samples should be sent for molecular confirmation. However to avoid unnecessary delay the counselling /clinical team must be contacted straight away that a couple possibly at risk for homozygous alpha thalassaemia has been identified. Do not wait for the DNA confirmation results to come back before alerting the counselling/clinical team that the couple should be offered counselling.

Q6: What do I do about alpha thalassaemia testing when one or both partners have mixed family origins?
A: If both parents' family origins include any ancestry from any area at high risk of alpha zero thalassaemia then alpha thalassaemia must be considered. These areas are identified with a red hash on the family origin questionnaire.

Beta thalassaemia

Q7: We are a high prevalence area and we have identified a lady who is of White British origin whose results suggest beta thalassaemia. Her partner is White British do we need to test him?
A: Yes, if a risk is identified in the mother then the baby's father should always be tested regardless of family origins. The exception to this is alpha thalassaemia where the family origins of both parents are considered.

Q8: We are a low prevalence area and we have been notified about a White British antenatal lady who has previously been identified outside the screening programme as being a carrier for beta thalassaemia. As White British ladies are not normally included in our programme, do we need to test her partner?
A: All women in England are offered antenatal thalassaemia screening. This includes areas designated as low and high prevalence for sickle cell screening. Yes, if a risk is identified or known about in the mother then the baby's father should always be tested regardless of the family origins of either parent. The exception to this is alpha thalassaemia where the family origins of both parents are considered.

Q9: I have a blood sample that we have run twice on our HPLC system. On one occasion the A2 level was 3.5% on the second it was 3.4%, the MCH is 26.5pg. I have 3 questions:
a) Should I test her partner?
b) Should I report her as beta thalassaemia trait?
c) Should I be re-running these borderline A2 levels on our HPLC system?
A:
a) You obtained an A2 value of 3.5% for this lady which is the screening programme action level when the MCH is below 27pg, therefore screening should be offered to the baby's father.
b) These parameters could be indicative of beta thalassaemia carrier which is why screening must be offered to the baby's father. However these indices are borderline and are not typical of beta thalassaemia carriers, therefore it is also possible the lady is normal with respect to beta thalassaemia. She should therefore be reported as a possible* beta thalassaemia carrier. DNA testing would be required to give a

NHS
National Institute for
Health Research

Summary

- Support service popular as offers immediate help and support to screening laboratories and other health professionals
- Excellent method for identifying educational and training needs
- Important tool for informing the screening programme about potential problems at ground level

Please Contact Us!

Designated Telephone line:
01865 572 769

Secure e-mail:-
lab.support@nhs.net