Leukaemia Diagnosis Interpretation

All Participants

Participant: **Trial No:** 232401

Date Issued: 24-May-2023 **Closing Date:** 14-June-2023

Trial Comments

This exercise was issued to 860 participants of which 418 (49%) returned results. There were no extension requests or pre-notified non-returns.

Your Diagnosis: AML with mutated NPM1

Consensus Diagnosis AML with mutated NPM1

Your Diagnosis is classed as being **CORRECT DIAGNOSIS (on rare occasions this may not be the consensus diagnosis).**

Performance Score: 0

Running Score: 50

Consensus

	Consensus Diagnosis	Your Diagnosis
Lineage	Myeloid Neoplasms	Myeloid Neoplasms
Subclassification	Acute myeloid leukemia (AML) and related precursor neoplasms	Acute myeloid leukemia (AML) and related precursor neoplasms
Diagnosis	AML with mutated NPM1	AML with mutated NPM1

Breakdown of returned diagnoses

Diagnosis	No Of Returns	Percentage Of Returns
Total Returns	418	100.0
AML with mutated NPM1	387	92.6
AML without maturation	12	2.9
AML with minimal differentiation	9	2.2
AML with maturation	3	0.7
Acute myelomonocytic leukaemia	2	0.5
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11	2	0.5
Acute monoblastic and monocytic leukaemia	1	0.2
AML with myelodysplasia-related changes	1	0.2
Further diagnosis cannot be made based on the information available	1	0.2

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Please note that we will be changing to the 5th Edition of the WHO guidelines after they become available. Diagnosis selection and the exercise report will be updated to reflect the changes in the 5th Edition. Participants will be notified of the details of the changes before they are implemented.

Clinical History/Information

A FBC performed as part of investigations on a 54-year-old woman admitted into hospital showed the following results- Hb 85 g/L; RBC 3.21 x10^12/L; WBC 320.17 x 10^9/L; PLT 72 x 10^9/L.

Bone marrow differential: Erythroid 0%; Myeloid 0%; Lymphoid 2%; Neutrophils <1%, Metamyelocytes 0%; Myelocytes 0%; Promyelocytes 0%; Blasts 98 %. Cells Counted 200

Immunophenotype

The consensus results from exercise LI 232401 showed that the malignant cells had the following phenotype (consensus antigen intensity expression in brackets):

Positive antigens: CD13 (Weak), CD33 (Strong), CD38 (Weak), CD45 (Weak), Myeloperoxidase (Strong)

Negative antigens: CD2 (Absent), CD3 (Absent), CD4 (Absent), CD7 (Absent), CD10 (Absent), CD11b (Absent), CD14 (Absent), CD15 (Absent), CD16 (Absent), CD19 (Absent), CD34 (Absent), CD56 (Absent), CD64 (Absent), CD117 (Absent), Cyto CD3 (Absent), HLA-DR (Absent), TDT (Absent)

Please note CD117 was reported positive by the referring laboratory.

Cytogenetics and Molecular genetics

PLEASE NOTE - Participants should be aware that, when submitting a diagnosis, if a specific pathology that has not been provided in the clinical details (e.g. molecular or cytogenetics) has been assumed to be present or introduced this will result in their diagnosis being classified as incorrect.

NPM1 **–** 4 bp duplication detected - NM_002520.7(NPM1):c.860_863dup**.**

FLT3 ITD **-** 27 bp ITD detected**;** *FLT3* TKD **-** No variant detected

FISH analysis of 100 interphase nuclei has shown no evidence of *PML*::*RARA*, *CBFB*::*MYH11* or *RUNX1*::*RUNX1T1* rearrangements.

Peripheral Blood Morphology- Please note that these comments are based on the clinical history, full blood count results, and digital image alone; no other results have been taken into consideration.

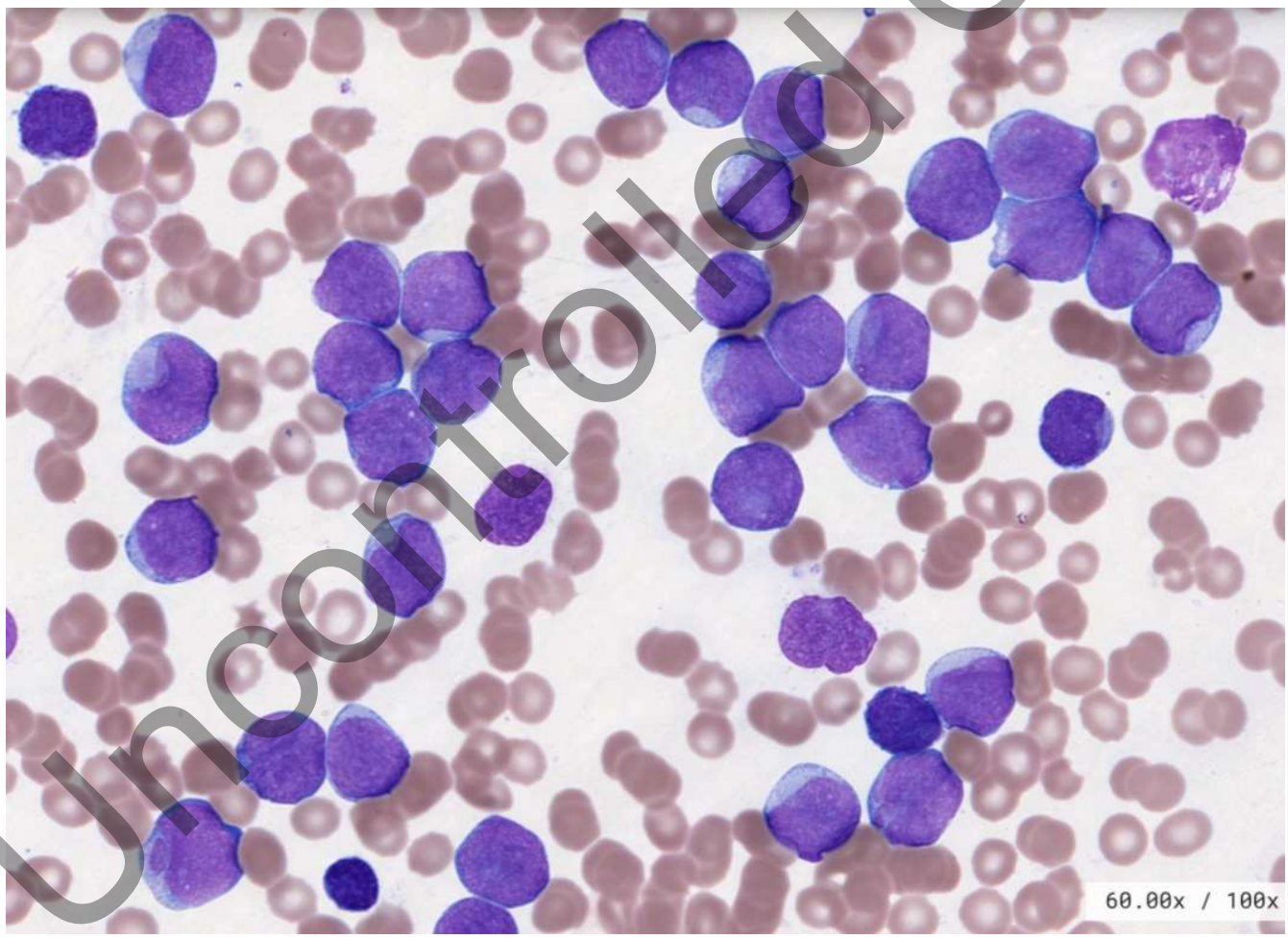
Morphology comments from Professor Wendy Erber
Consultant Haematologist, University of Western Australia, Nedlands, Australia

Blood film: There is a marked leucocytosis, with the cells being blast cells. These are of intermediate size with a very high nuclear:cytoplasmic ratio. The nuclei are round and some indented, or with convolutions and with “cup-like” appearance. The chromatin is fine and many blast cells have a nucleolus. The cytoplasm is basophilic, and granules are present in a small percent of the blast cells. Small vacuoles are noted, and rare Auer rods are seen. There is accompanying neutropenia and thrombocytopenia.

COMMENT: Acute myeloid leukaemia. The cup-like morphology is associated with, but not diagnostic of, acute myeloid leukaemia, *NPM1* mutated.

Example of Digital Image Issued

Figure 1: Peripheral Blood x60 magnification (May Grünwald Giemsa stain)



Exercise Conclusion/Case Discussion

Please note that this trial requires a diagnosis based on the WHO guidelines. This report is therefore based on the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Revised 4th Edition 2017[1]. *Please note that we will be changing to the 5th Edition of the WHO guidelines after they become available. Diagnosis selection and the exercise report will be updated to reflect the changes in the 5th Edition. Participants will be notified of the details of the changes before they are implemented.*

Findings from Leukaemia Immunophenotyping - Part One

There was generally good consensus for the commonly tested antigens with CD117 being the exception. 267 participants submitted results for CD117 with the majority reporting it as negative (149 participants). However, 118 participants reported it as positive. Analysis did not show effect of a specific clone, fluorochrome or antibody manufacturer. As a result, due to the lack of clear consensus, UK NEQAS LI took the decision to not include CD117 as one of the top 10 antigens and to exclude it from scoring. It was replaced with CD64 which is 11th on the list of most tested for antigens. Of the 149 participants that reported CD117 as negative, 146 noted the intensity as absent and 3 noted it as weak. Out of the 118 participants that reported it as positive, 1 noted the intensity as absent, 96 reported the intensity as weak and 21 reported it as strong.

Please note that CD117 was reported positive by the referring laboratory.

Further, data analysis was performed to see if any laboratories used the same panel. This analysis found that there were 269 panels being used between 288 laboratories. Five panels were identified as being used by multiple sites, meaning that 93% of sites were using unique/bespoke panels.

- One panel was used by 5 laboratories: Three in Switzerland, and one each in Romania and Uruguay.
- Another panel was used by 3 laboratories, all in Malaysia.
- Two laboratories, both in Poland used the same panel.
- Two laboratories, one in Chile and the other in Portugal used the same panel.
- Two laboratories, one in France and the other in Sweden used the same panel.

Participants are scored based on the number of the top 10 tested antigens they tested for and the number of those with results in consensus. For this exercise, 65.4% (189/289) of participants who submitted results tested for all the antigens in the top 10. Out of these, 30.2% (57/189) had all ten results in consensus; 32.3% (61/189) had 9 out of 10 results in consensus; 22.2% (42/189) had 8 out of 10 results in consensus; 10.1% (19/189) had 7 out of 10 results in consensus; 4.2% (8/189) had 6 out of 10 results in consensus and 1.1% (2/189) had 5 out of 10 results in consensus.

Leukaemia Diagnostic Interpretation - Part Two

Consensus Diagnosis

AML with mutated *NPM1*

Correct Diagnoses

AML with mutated *NPM1* is one of the most common and relatively specific mutations in AML and is more commonly seen in women. It is present in between 2% and 8% of childhood cases, between 27% and 35% of adult cases and between 45% and 64% of cases in adults with a normal karyotype. Patients often present with anaemia and thrombocytopenia and higher white cell and platelet counts than seen in other types of AML. Extramedullary site involvement can occur e.g., the skin, the gingiva and lymph nodes.

The *NPM1* mutation is often seen in cases of acute myelomonocytic leukaemia and acute monocytic leukaemia. It is also seen in AML with maturation and AML without maturation as well as pure erythroid leukaemia. Dysplasia is seen in multiple lineages in up to 25% of *de novo* AML with mutated *NPM1.*

Immunophenotypically there is strong expression of CD33 and variable expression of CD13. Expression of CD117, CD123 and CD110 is common but HLADR tends to be negative. Two main subgroups of AML with mutated *NPM1* have been reported: one with a monocytic immunophenotypic profile (positive for CD14, CD36 and CD64) and the other with an immature myeloid profile. CD34 is often negative but when positive, it is often associated with an adverse prognosis.

NPM1 mutation is usually associated with a normal karyotype although chromosomal changes such as the gain of chromosome 8 and del(9q) are detected in 5% to 15% of cases. Del(9q) is considered a myelodysplasia-related abnormality in the majority of AMLs but not when a mutation of *NPM1* is present. Secondary mutations are common with *NPM1* mutations, with mutations of *FLT3* and *DNMT3A* being the most frequently seen.

Based on the morphology, immunophenotype and detection of the *NPM1* mutation, the diagnosis of AML with mutated *NPM1* is the only correct diagnosis.

Differential Diagnoses

None for this exercise

Incorrect Diagnoses

AML without maturation is classed as incorrect because it fails to take into account the presence of the *NPM1 mutation.* In addition, the immunophenotype does not match that of this case.

AML with minimal differentiation is classed as incorrect because it fails to take into account the presence of the *NPM1* mutation. In addition, the immunophenotype does not match that of this case.

AML with maturation is classed as incorrect because it fails to take into account the presence of the *NPM1* mutation. In addition, the immunophenotype and morphology do not match that of this case.

Acute myelomonocytic leukaemia is classed as incorrect because it fails to take into account the presence of the *NPM1* mutation. In addition, the immunophenotype and morphology do not match that of this case.

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB*::*MYH11* is classed as incorrect because it fails to take into account the presence of the *NPM1* mutation. In addition, it assumes the presence of a genetic abnormality which is not found in this case. The immunophenotype does not match that of this case either.

Acute monoblastic and monocytic leukaemia is classed as incorrect because it fails to take into account the presence of the *NPM1* mutation. In addition, the immunophenotype and morphology do not match that of this case.

AML with myelodysplasia-related changes is classed as incorrect because it fails to take into account the presence of the *NPM1* mutation. In addition, the morphology does not match that of this case.

Further diagnosis cannot be made based on the information available is classified as incorrect as selecting this option fails to take into account the presence of the *NPM1* mutation. There is sufficient information provided with this case for the correct diagnosis to be made.

Further Trial Findings

Table 1: Submissions from all participants - laboratories and individuals

Diagnosis	Total No. of Returns	No. of Laboratories	No. of Individuals
AML with mutated *NPM1*	387	233	154
AML without maturation	12	9	3
AML with minimal differentiation	9	4	5
AML with maturation	3	1	2
Acute myelomonocytic leukaemia	2	1	1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB*::*MYH11*	2	2	0
Acute monoblastic and monocytic leukaemia	1	0	1
AML with myelodysplasia-related changes	1	1	0
Further diagnosis cannot be made based on the information available	1	1	0

Table 2: Submissions from laboratory participants only

Diagnosis	Laboratory Returns %
AML with mutated *NPM1*	92.5
AML without maturation	3.6
AML with minimal differentiation	1.6
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB*::*MYH11*	0.8
AML with maturation	0.4
Acute myelomonocytic leukaemia	0.4
AML with myelodysplasia-related changes	0.4
Further diagnosis cannot be made based on the information available	0.4

Table 3: Submissions from individual participants only

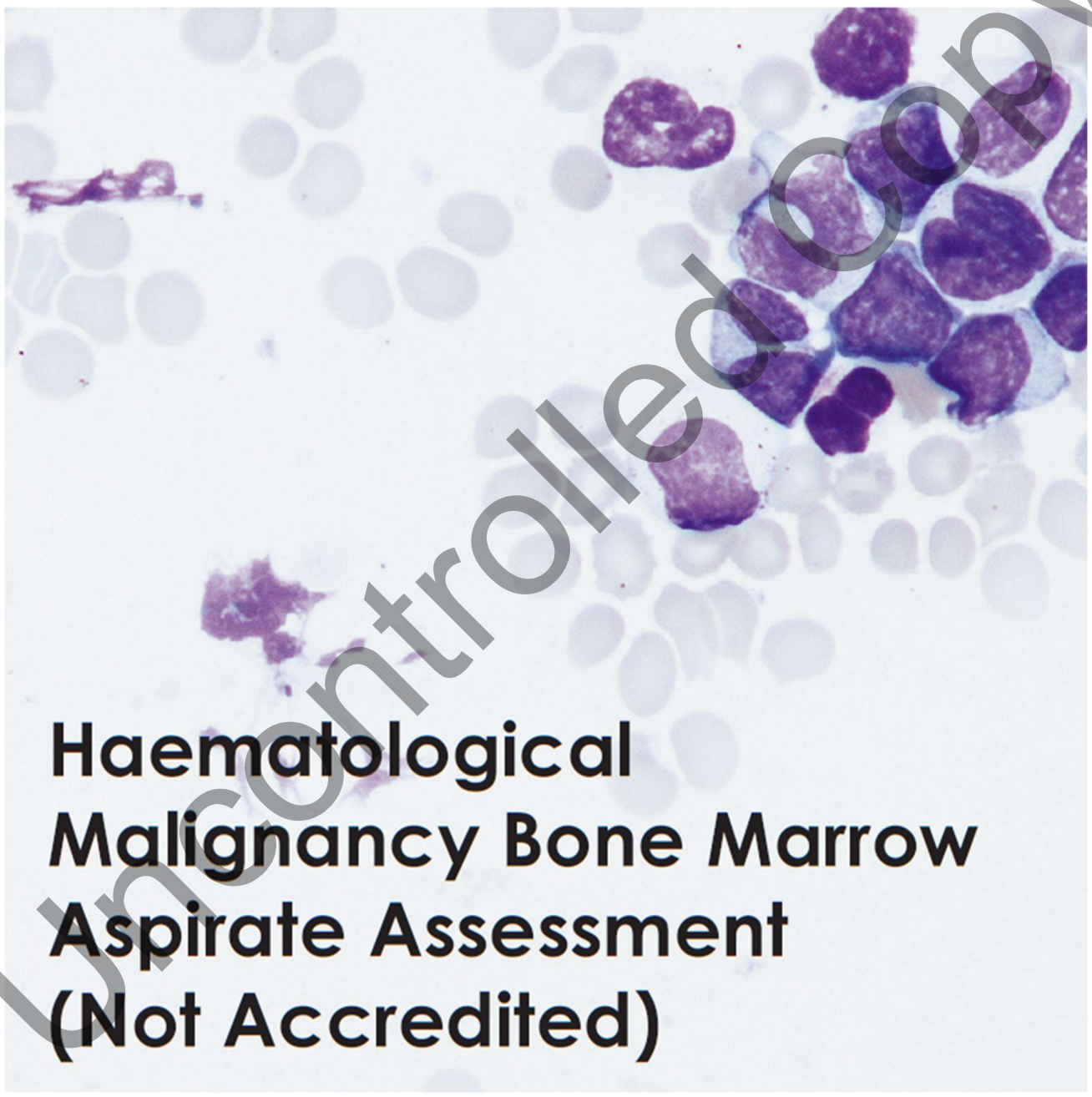
Diagnosis	Individual Returns %
AML with mutated *NPM1*	92.8
AML with minimal differentiation	3.0
AML without maturation	1.8
AML with maturation	1.2
Acute myelomonocytic leukaemia	0.6
Acute monoblastic and monocytic leukaemia	0.6

Reference:
1. Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S Jaffe, Stefano A. Pileri, Harald Stein, Jürgen Thiele, Daniel A. Arber, Robert P. Hasserjian, Michelle M. Le Beau, Attilio Orazi and Reiner Siebert. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th Edition. IARC Press 2017

NOTICE: DO YOU OR ANY OF YOUR COLLEAGUES ASSESS BONE MARROW MORPHOLOGY? DID YOU KNOW THAT WE ALSO RUN A DIGITAL BONE MARROW ASPIRATE PROGRAMME FOR HAEMATO-ONCOLOGY? FOR FURTHER DETAILS OR TO JOIN PLEASE VISIT OUR WEBSITE WWW.UKNEQASLI.CO.UK OR EMAIL ADMIN@UKNEQASLI.CO.UK.THIS IS LOW COST AND CAN COUNT TOWARDS YOUR CONTINUED PROFESSIONAL DEVELOPMENT (CPD) PORTFOLIO.

UK NEQAS

Leucocyte Immunophenotyping

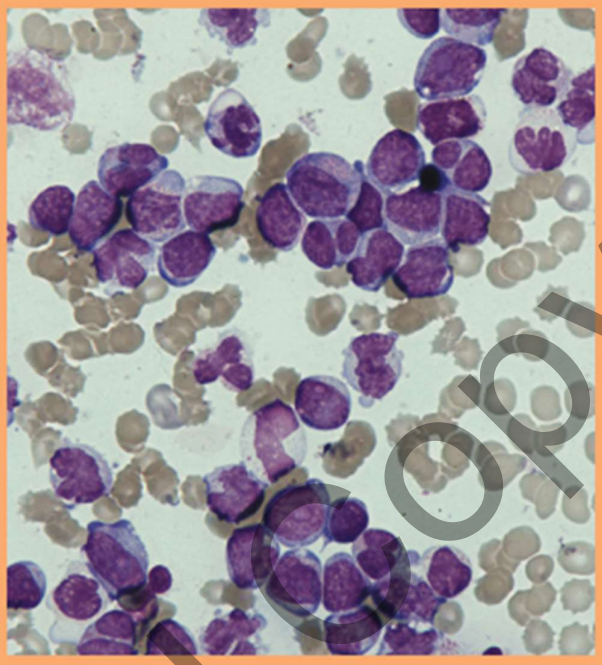


Haematological Malignancy Bone Marrow Aspirate Assessment (Not Accredited)

Haematological Malignancy Bone Marrow Aspirate Assessment (Not Accredited)

This programme is designed to assess the ability of participants to identify cell types in a bone marrow aspirate.

Sheffield Teaching Hospitals NHS Foundation Trust, a UKAS proficiency testing provider No. 7804, operating UK NEQAS for Leucocyte Immunophenotyping.



Scheme	Examination	Sample format	Distribution per year	Number of samples per distribution	Scoring
Haematological Malignancy Bone Marrow Aspirate Assessment **(Not Accredited)**	Digital morphology	Web based programme Digital bone marrow image	4	Web based digital image	To be determined

Should you wish to participate in the new programme or any of the existing ones, please contact*

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United Kingdom

Tel: +44 (0)114 2673600
Fax: +44 (0)114 2673601
email: admin@ukneqasli.co.uk
www.ukneqasli.co.uk

*Please note that if your participation with UK NEQAS LI is via a distributor then you should contact your distributor for details of how to register.

Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600
e-mail: amanda.newbould@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Mr Liam Whitby (Director) and Mr Stuart Scott (Centre Manager).

4.8.2 c) Person(s) authorizing this report:
Mr Liam Whitby (Director) or Mr Stuart Scott (Centre Manager) of UK NEQAS LI.

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance (for BS EN ISO/IEC 17043:2010 accredited programmes only). Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided. Alternatively, if you are unhappy with your performance classification for this trial, please find the appeals procedure at www.ukneqasli.co.uk/contact-us/appeals-and-complaints/

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