Newsletter

UK NEQAS
International Quality Expertise

Autore: Dr. Bruno Brando
Data: 7 Giugno 2022
Riferimento: UK NEQAS LI

OGGETTO: suggestioni dal "12° ITALIAN UK NEQAS USERS MEETING Diagnostica oncoematologica ed immunologica in citometria" – 5 Maggio 2022


EVENTO WEBINAR

UK NEQAS
International Quality Expertise

12° ITALIAN UK NEQAS LI USERS MEETING

Diagnostica oncoematologica ed immunologica in citometria

Giovedì 5 Maggio 2022

Il 12° UK NEQAS LI Users Meeting, un appuntamento ormai consueto che riunisce i Laboratori che aderiscono ai programmi di External Quality Assessment/Proficiency Testing di UK NEQAS for Leucocyte Immunophenotyping.
Si tratta di un importante momento di condivisione e discussione sulle problematiche emerse durante l'esecuzione degli esercizi dell'ultimo anno e un'occasione di aggiornamento su come stanno cambiando gli scenari diagnostici e l'organizzazione dei laboratori italiani, anche in confronto alle nuove esigenze dei sistemi qualità e dei processi di accreditamento.
Al fine di poter conseguire i crediti ECM previsti, l'utente dovrà completare il test che verrà messo a disposizione al termine dell'incontro.

PROGRAMMA

h. 14:30 Apertura evento e collegamento partecipanti

h. 14:50 Introduzione ai lavori e obiettivi del corso
Bruno Brando (Milano)

h. 15:00 Immune Monitoring dei linfociti B nei pazienti trattati con anticorpi Anti CD20
Arianna Gatti (Legnano)

h. 15:30 Analisi citometrica del liquor e criteri standard di lettura
Maria Ilaria Del Principe (Roma)

h. 16:00 Programma UK NEQAS per l'identificazione delle cellule patologiche nell'agoaspirato midollare
Anna Maria Pollono (Torino)

h. 16:30 Discussione

h. 17:00 Chiusura lavori

Segreteria Organizzativa
Provider ECM n° 459

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REGISTRAZIONE E ACCESSO

La registrazione gratuita e l'accesso al corso potranno essere effettuate collegandosi al link: https://fad.summeet.it

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- Una volta effettuata l'iscrizione, potrà accedere alla pagina del corso e frequentarlo

Per eventuali problematiche di accesso, potrà contattare l'help desk fad@summeet.it - 0332 231416

Introduzione ai lavori - Dr. Bruno Brando: Gli argomenti del webinar del 5/5/2022 hanno riguardato importanti progressi nelle applicazioni delle tecniche citometriche e nella diagnostica citomorfologica di interesse oncoematologico.
Il 'Monitoraggio Immunologico (Immune Monitoring)' così come siamo abituati a definirlo sta oggi subendo un'importante trasformazione, estendendosi all'analisi delle popolazioni funzionali dei linfociti B, all'analisi citometrica degli eventi infettivi e della sepsi, per finire nel campo ancora poco esplorato dei trattamenti con cellule CAR-T. Queste nuove prospettive mettono ulteriormente alla prova il laboratorio di citometria, che oggi più che mai richiede il supporto di rigorosi programmi di controllo del processo analitico.
Dopo quasi 15 anni dalla pubblicazione delle prime linee-guida internazionali, un gruppo di lavoro ISCCA/ESCCA ha profondamente rivisto le tecniche citometriche per l'analisi del liquido cefalo-rachidiano di interesse leucemologico. Sono state stabilite nuove regole operative per l'analisi citometrica del liquor, definendo innovativi criteri analitici, che rendono più robusta e affidabile questa delicata tecnica. Su queste basi è in corso di revisione e aggiornamento lo schema pilota UK NEQAS LI.
L'analisi citomorfologica dell'agoaspirato midollare sembra da qualche tempo svolgere il ruolo della Cenerentola del percorso diagnostico delle malattie oncoematologiche, per le straordinarie performance delle tecniche analitiche più sofisticate e moderne, inclusa la citometria. Questa analisi rimane tuttavia un passaggio ineludibile nella diagnostica, purtroppo con sempre meno persone dotate della necessaria esperienza e pazienza, disponibili a praticarla in modo continuativo e affidabile. Lo schema UK NEQAS LI Bone Marrow Aspirate Assessment, di recente apertura, è stato sviluppato per educare gli operatori ad una pratica di lettura standardizzata e quanto più possibile oggettiva dell'agoaspirato midollare, grazie alla disponibilità di ottime immagini digitali ad alta risoluzione e di checklist per una valutazione sistematica di tutte le componenti cellulari.


EVENTO WEBINAR

UK NEQAS
International Quality Expertise

12° ITALIAN UK NEQAS LI USERS MEETING

Diagnostica oncoematologica ed immunologica in citometria

Giovedì 5 Maggio 2022

Immune monitoring dei linfociti B nei pazienti trattati con anticorpi anti CD20

Arianna Gatti
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Immune Monitoring dei linfociti B nei pazienti trattati con anticorpi Anti-CD20 - Dr.ssa Arianna Gatti: Nei decenni passati l'analisi della risposta immune ed il monitoraggio dei trattamenti con farmaci immunosoppressori erano incentrati sulla linea T linfocitaria. Con la scoperta quasi casuale che l'anticorpo monoclonale Rituximab (anti-CD20) utilizzato nelle malattie linfoproliferative B otteneva sorprendenti effetti curativi nell'artrite reumatoide si è aperto un nuovo scenario di applicazioni terapeutiche nelle malattie autoimmuni. Sono infatti oggi molto numerose le patologie che possono venire trattate con anticorpi anti-CD20. Questo ha tuttavia generato la necessità di sviluppare uno specifico schema di monitoraggio immunologico centrato sulla linea B linfocitaria. Il monitoraggio è diretto a valutare il corretto meccanismo d'azione del farmaco (per identificare i pazienti resistenti), il corretto processo di deplezione B con tecniche citometriche ad alta sensibilità e la tempistica e qualità del ripopolamento, che se operato da linfociti B naïve CD27-negativi costituisce un indicatore di risposta clinica efficace. Lo scopo è quello di fornire ai clinici elementi oggettivi per ottimizzare gli schemi di trattamento, osservando la dinamica di questi nuovi indicatori biologici dal comportamento marcatamente individuale. Il protocollo citometrico ISCCA a 8 colori e 10 marcatori permette di identificare ed enumerare ad alta sensibilità tutti i più rilevanti subset funzionali B ed il loro grado di differenziamento, assieme alla contemporanea valutazione di linfociti T e NK. UK NEQAS LI farà circolare un questionario per valutare la diffusione di questa nuova metodica, allo scopo di introdurre l'analisi dei subset funzionali B nello schema Immune Monitoring, per i centri impegnati a svolgere il monitoraggio dei trattamenti con anticorpi anti-CD20.

Analisi citometrica del liquor e criteri standard di lettura - Dr.ssa Maria Ilaria Del Principe: Lo studio citometrico del liquor è un importante elemento nella diagnostica e nel follow-up delle leucemie acute linfoblastiche, dei linfomi non-Hodgkin e delle leucemie mieloidi acute. Contrariamente all'analisi del liquor nei sospetti di meningiti da agenti infettivi, l'ambito diagnostico è qui focalizzato sulla valutazione della numerosità e sul riconoscimento di cellule patologiche, in un contesto spesso ricco di elementi confondenti, cellule infiammatorie e detrito. L'analisi in citologia convenzionale (cytospin) possiede un buon grado di specificità ma una scarsa sensibilità (<50%), in contrasto con l'analisi citometrica, che può superare l'80%. La scarsità di cellule, spesso meno di 5-10 per microlitro, non gestibile dai comuni contaglobuli, è invece facilmente e accuratamente valutabile in citometria con l'uso di CD45 e microsfere di conteggio. Come è noto occorre lavorare rapidamente, in modo gentile, acquisendo il maggior numero possibile di eventi e massimizzando l'informazione con l'impiego di uno o massimo due tubi a 6-8 colori. Importante è una chiara valutazione del cluster citometrico di cellule mononucleate, che spesso indirizza verso la possibilità di trovare nel suo contesto gli elementi patologici. Controversie ancora esistono sui cutoff numerici per definire positività o negatività. Il position paper ESCCA recentemente pubblicato analizza e discute tutti gli aspetti tecnici rilevanti dell'analisi citometrica del liquor e costituisce il nuovo standard operativo sul quale si sta allineando anche lo schema UK NEQAS LI in via di perfezionamento.

Programma EQA/PT UK NEQAS LI per l'Immunofenotipizzazione delle cellule del Liquor

Questo programma è stato sviluppato per stabilire la capacità dei laboratori di identificare e caratterizzare immunofenotipicamente in citometria a flusso cellule leucemiche in campioni di Liquor.
È previsto un campione di leucociti stabilizzati risospesi in un medium che riproduce le caratteristiche del liquor, assieme ad un'immagine digitale di un citospin, utile per una valutazione morfologica preliminare all'analisi fenotipica.
Ai partecipanti sarà richiesto di analizzare il campione utilizzando le proprie metodologie di routine, per definire l'eventuale presenza di un certo tipo di contaminazione leucemica. Saranno raccolti i dati di conteggio cellulare assoluto, di valutazione morfologica e di analisi immunofenotipica in citometria.

esempio di report allegato alla presente Newsletter

Programma UK NEQAS per l'identificazione delle cellule patologiche nell'agoaspirato midollare - Dr.ssa Anna Maria Pollono: Grazie alla disponibilità di nuove e chiare immagini ad altissima risoluzione e facili comandi è oggi possibile analizzare al computer un agoaspirato midollare come se fossimo seduti davanti al nostro microscopio (e magari con una visuale anche migliore). Lo schema UK NEQAS è relativamente giovane e ancora non molto conosciuto in Italia, ma costituisce un'opportunità educazionale unica per cercare di raggiungere il migliore consenso possibile in una materia notoriamente tarata di un alto grado di soggettività e variabilità tra diversi operatori. Ai partecipanti viene richiesto di lavorare secondo una check-list che richiede la valutazione qualitativa e quantitativa di tutte le componenti cellulari, normali e patologiche, secondo le regole classiche dei maestri dell'ematologia Dacie & Lewis. Chi lavora in questo campo sa quanto sia impegnativo e a volte tedioso analizzare in modo approfondito un agoaspirato midollare, e l'utilizzo della check-list facilita e sveltisce grandemente questo compito, valorizzando solo le informazioni oggi ritenute più rilevanti. L'esercizio finisce con la richiesta di classificare correttamente cinque cellule pre-marcate con tag, scegliendo in menu a tendina. E qui cominciano i dolori: dai report si evidenzia con sconcerto come il consenso sul riconoscimento di elementi cellulari - anche delle normali linee emopoietiche - sia a volte drammaticamente scarso. È una ragione in più per applicarsi in questa materia, consapevoli che non sempre l'analisi citometrica fornisce tutte le risposte e che un buon citometrista deve maturare anche competenze di citomorfologia, magari affrontando gli esercizi dello schema UK NEQAS con l'assistenza dei colleghi patologi.

Programma EQA/PT UK NEQAS LI per l'analisi Citomorfologica dell'Agoaspirato Midollare

Il programma è interamente telematico: in ogni esercizio verrà proposta un'immagine ad alta risoluzione, nella quale si dovrà dare una valutazione citomorfologica dell'aspirato, si dovrà fornire il conteggio differenziale globale ed il conteggio della percentuale di blasti, sul totale degli elementi nucleati. Si dovranno inoltre classificare cellule pre-identificate da marcatori e fornire indicazioni su eventuali test aggiuntivi da eseguire a seguito della valutazione morfologica.

esempio di report allegato alla presente Newsletter

La nostra speranza è che il prossimo 13° ITALIAN UK NEQAS USERS MEETING si potrà tenere in presenza, così da poter stimolare il confronto e il dibattito che sono sempre state le peculiarità degli incontri annuali UK NEQAS LI.

Dr. Bruno Brando
Referente scientifico UK NEQAS for Leucocyte Immunophenotyping

CASELLA DI POSTA PER INFORMAZIONI SCIENTIFICHE: supporto.tecnico@flowassessment.it

Presentazioni 12° ITALIAN UK NEQAS USERS MEETING
Diagnostica oncoematologica ed immunologica in citometria

Le presentazioni sono disponibili online e scaricabili dal sito di FLOW ASSESSMENT

www.flowassessment.it -> EVENTI -> ARCHIVIO EVENTI -> UK NEQAS LI – 12° UK NEQAS LI USERS MEETING -> PRESENTAZIONI
Link: https://www.flowassessment.it/eventi/uk-neqas-li-12-uk-neqas-li-users-meeting-citometria/

PASSWORD: WEBINAR22

Bibliografia:

Gatti A, Buccisano F, Scupoli MT, Brando B. The ISCCA flow protocol for the monitoring of anti-CD20 therapies in autoimmune disorders. Cytometry Part B Clin. Cytom. 2021; 100: 194-205.

Del Vecchio L, Allinovi M, Rocco P, Brando B. Rituximab Therapy for Adults with Nephrotic Syndromes: Standard Schedules or B Cell-Targeted Therapy? J Clin Med. 2021 Dec 13; 10(24): 5847. doi: 10.3390/jcm10245847.

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Parmentier S, Schetelig J, Lorenz K, Kramer M, Ireland R, et al. Assessment of dysplastic hematopoiesis: lessons from healthy bone marrow donors. Haematologica 2012; 97(5): 723-730.

Senent L, Arenillas L, Luño E, Ruiz JC, Sanz G and Florensa L. Reproducibility of the World Health Organization 2008 criteria for myelodysplastic syndromes. Haematologica 2013; 98(4): 568-575.

Parmentier S, Kramer M, Weller S, Schuler U, Ordemann R, Rall G, et al. Reevaluation of reference values for bone marrow differential counts in 236 healthy bone marrow donors. Annals of Hematology 2020; 99: 2723–2729.

i lavori qui elencati sono richiedibili a supporto.tecnico@flowassessment.it

UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Cerebrospinal Fluid (CSF) Immunophenotyping (Not Accredited)

All Participant Report

Distribution – 212202 **Sample - 009**

Date Issued – 31/03/2022 **Closing Date –** 20/04/2022

PLEASE NOTE – THIS PROGRAMME IS NOT CURRENTLY PERFORMANCE MONITORED

Trial Comments

This trial was issued to 47 participants. Results were returned by 38 participants.

Please note that this is a pilot programme and is therefore not subject to performance monitoring. All information shown in this report is provided for information purposes only.

Sample Comments

The CSF sample comprised of suspended stabilised white blood cells from a 32-year-old female previously in B-ALL remission. Querying relapse of disease and CNS involvement.

Haematology Analyser results –

	WBC	RBC
Peripheral Blood	13.87 x 10^9L	3.68 x 10^{12}L
CSF	37 cells/µL	1000 cells/µL

With reference to the appearance of the sample, is any red cell contamination visible?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	No	No	24/38 (63.2%)
		Yes	14/38 (36.8%)

Did you examine the morphology of the cytospin image?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Yes	Yes	37/38 (97.4%)
		No	1/38 (2.6%)

Are a large number of red blood cells present suggesting a traumatic tap?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	No	No	34/38 (89.5%)
		Yes	4/38 (10.5%)

Were reactive /malignant cells observed?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Not reached	Malignant cells observed	15/38 (39.5%)
		Reactive cells observed	12/38 (31.6%)
		No abnormal cells observed	11/38 (28.9%)

Following examination of the cytospin, is immunophenotyping recommended?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Yes	Yes	34/38 (89.5%)
		No	4/38 (10.5%)

Following immunophenotyping was a discreet population of malignant cells identified?

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	No	No	27/38 (71.1%)
		Yes	7/38 (18.4%)
		N/A	4/38 (10.5%)

Conclusion of CSF investigations

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Not reached	No CNS involvement of disease	18/38 (47.4%)
		CNS involvement of disease	14/38 (36.8%)
		Reactive Lymphocytosis	6/38 (15.8%)

Trial Summary

- This sample was produced to mimic a patient with CNS involvement of disease
- No consensus conclusion was reached concerning whether or not there was CNS involvement of disease
- The lack of red blood cell contamination of the sample and the fact that no red blood cells were seen in the cytospin suggested that the tap was not traumatic
- Immunophenotyping was reported to be required by 34/38 of participants with 27 of those participants reporting no discreet population of malignant cells.
- This sample did prove to be more challenging. We were able to create a suitable cytospin image, but a small number of participants reported a difficulty in obtaining suitable immunophenotyping results

Cerebrospinal Fluid (CSF) Immunophenotyping (Not Accredited)

All Participant Report

Distribution – 212202 **Sample - 010**

Date Issued – 31/03/2022 **Closing Date –** 20/04/2022

PLEASE NOTE – THIS PROGRAMME IS NOT CURRENTLY PERFORMANCE MONITORED

Trial Comments

This trial was issued to 47 participants. Results were returned by 38 participants.

Please note that this is a pilot programme and is therefore not subject to performance monitoring. All information shown in this report is provided for information purposes only.

Sample Comments

The CSF sample comprised of suspended stabilised white blood cells from 41-year-old male with a history of lymphoma. Querying relapse of disease and CNS involvement.

Haematology Analyser results –

	WBC	RBC
Peripheral Blood	6.01 x 10^9L	4.72 x 10^{12}L
CSF	14 cells/µL	3000 cells/µL

With reference to the appearance of the sample, is any red cell contamination visible?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Yes	Yes	23/38 (60.5%)
		No	15/38 (39.5%)

Did you examine the morphology of the cytospin image?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Yes	Yes	38/38 (100.0%)

Are a large number of red blood cells present suggesting a traumatic tap?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Yes	Yes	36/38 (94.7%)
		No	2/38 (5.3%)

Were reactive /malignant cells observed?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	No abnormal cells observed	No abnormal cells observed	32/38 (84.2%)
		Reactive cells observed	6/38 (15.8%)

Following examination of the cytospin, is immunophenotyping recommended?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Not reached	No	20/38 (52.6%)
		Yes	18/38 (47.4%)

Following immunophenotyping was a discreet population of malignant cells identified?

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	No	No	31/38 (81.6%)
		N/A	6/38 (18.4%)

Conclusion of CSF investigations

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	No CNS involvement of disease	No CNS involvement of disease	35/38 (92.1%)
		Reactive Lymphocytosis	3/38 (7.9%)

Trial Summary

- This sample was produced to mimic a traumatic tap
- The overall consensus was that there was no CNS involvement of disease however 3/38 participants reported the presence of reactive lymphocytes
- Red cell contamination of the sample was reported by 23/38 of participants and 36/38 reported that red blood cells were seen in the cytospin suggesting that the tap was traumatic
- There was almost a 50/50 split with regards to whether immunophenotyping was required
- Although 18 participants suggested that immunophenotyping was not required, 31/38 participants reported that no discreet population of malignant cells was identified

References

1. Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S Jaffe, Stefano A. Pileri, Harald Stein, Jürgen Thiele, Danial 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

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
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United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
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/

4.8.4) The UK NEQAS LI Policy for the Use of Reports by Individuals and Organisations states that all EQA reports are subject to copyright, and, as such, permission must be sought from UK NEQAS LI for the use of any data and/or reports in any media prior to use. See associated policy on the UK NEQAS LI website: http://www.ukneqasli.co.uk/eqa-pt-programmes/new-participant-information/

UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Haematological Malignancy Bone Marrow Aspirate Assessment (Not Accredited)
All Participant Report

Distribution – 212204 **Participant**

Date Issued – 30/03/2022 **Closing Date –** 26/04/2022

Trial Comments

This trial was issued to 191 participants. Results were returned by 64 participants. This is the final version of the report.

Please refer to page10 – **Participant Instructions for Viewing Precipoint Scanned Images for The Haematological Malignancies Bone Marrow Aspirate Assessment Programme**

Link for the image - https://preci.cloud/slides/84b28a3f-6946-45db-b6e3-019863fadef7

Sample Comments

A scanned bone marrow aspirate from a 2-year-old female with Fanconi Anaemia with associated MDS-EB.

Breakdown of Bone Marrow Aspirate Interpretation and Further Testing

Cellular Identification Results

Cell A – Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Pelger–Huët	Pelger–Huët	39/64 (60.9%)
		Neutrophil	23/64 (35.9%)
		Blast	1/64 (1.6%)
		None of the above	1/64 (1.6%)

Cell B – Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Nucleated Red Blood Cell (All stages except Proerythroblast)	Nucleated Red Blood Cell (All stages except Proerythroblast)	35/64 (54.7%)
		Proerythroblast	23/64 (35.9%)
		Plasma Cell	4/64 (6.3%)
		Myelocyte	1/64 (1.6%)
		None of the above	1/64 (1.6%)

Cell C – Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Not Reached	Promonocyte	18/64 (28.1%)
		Blast	12/64 (18.8%)
		Myelocyte	12/64 (18.8%)
		Promyelocyte	12/64 (18.8%)
		Monocyte	4/64 (6.3%)
		Lymphocyte	2/64 (3.1%)
		Metamyelocyte	2/64 (3.1%)
		None of the above	2/64 (3.1%)

Cell D – Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Neutrophil	Neutrophil	38/64 (59.4%)
		Metamyelocyte	18/64 (28.1%)
		None of the above	5/64 (7.8%)
		Promonocyte	2/64 (3.1%)
		Blast	1/64 (1.6%)

Cell E – Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
	Large Granular Lymphocyte	Large Granular Lymphocyte	47/64 (73.4%)
		Lymphocyte	8/64 (12.5%)
		Monocyte	3/64 (4.7%)
		Myelocyte	3/64 (4.7%)
		None of the above	2/64 (3.1%)
		Nucleated Red Blood Cell (All stages except Proerythroblast)	1/64 (1.6%)

Bone Marrow Target Population Differential

Differential Results - Total number of nucleated cells counted

Your Response	Participant Responses	Number of Returns (Percentage value in brackets)
	1 - 100	14/64 (21.9%)
	101 - 200	23/64 (35.9%)
	201 - 300	14/64 (21.9%)
	301 - 400	2/64 (3.1%)
	401 - 500	10/64 (15.6%)
	501 - 600	1/64 (1.6%)

Differential Results - Percentage of blast cells in the bone marrow aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	1% – 20%	1% – 20%	49/64 (76.6%)
		21% – 40%	14/64 (21.9%)
		71% – 90%	1/64 (1.6%)

Please assess the bone marrow image and provide comment on the cellularity of the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Increased	Increased	33/64 (51.6%)
		Normal	25/64 (39.1%)
		Decreased	6/64 (9.4%)

Please assess and provide comment on megakaryopoiesis within the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Decreased	Decreased	50/64 (78.1%)
		Not seen	9/64 (14.1%)
		Normal	5/64 (7.8%)

Please state if megakaryocytic dysplasia is present and at what level

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Not Reached	Not present	27/64 (42.2%)
		Present at between 10% and 50%	16/64 (25.0%)
		Present at greater than 50%	12/64 (18.8%)
		Present at less than 10%	9/64 (14.1%)

Please assess and provide comment on erythropoiesis within the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Normal	Normal	38/64 (59.4%)
		Decreased	19/64 (29.7%)
		Increased	7/64 (10.9%)

Please state if erythroid dysplasia is present and at what level

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Present at between 10% and 50%	Present at between 10% and 50%	34/64 (53.1%)
		Present at greater than 50%	12/64 (18.8%)
		Present at less than 10%	11/64 (17.2%)
		Not present	7/64 (10.9%)

Please assess and provide comment on myelopoiesis within the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Increased	Increased	38/64 (59.4%)
		Normal	17/64 (26.6%)
		Left shifted	5/64 (7.8%)
		Decreased	4/64 (6.3%)

Please state if myelodysplasia is present and at what level

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Present at between 10% and 50%	Present at between 10% and 50%	35/64 (54.7%)
		Present at greater than 50%	19/64 (29.7%)
		Present at less than 10%	6/64 (9.4%)
		Not present	4/64 (6.3%)

Please assess and provide comment on the blasts within the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Increased	Increased	56/64 (87.5%)
		Normal	8/64 (12.5%)

Please comment on the maturation of the myeloid series within the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Left shifted	Left shifted	40/64 (62.5%)
		Normal	21/64 (32.8%)
		Arrested	3/64 (4.7%)

Please assess and provide comment on the lymphocyte numbers within the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Normal	Normal	51/64 (79.7%)
		Increased	7/64 (10.9%)
		Decreased	6/64 (9.4%)

Please assess and provide comment on the percentage of lymphocyte cells within the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	11% – 20%	1% – 10%	23/64 (35.9%)
		11% – 20%	33/64 (51.6%)
		21% – 30%	8/64 (12.5%)

Please assess and provide comment on the plasma cells within the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Normal	Normal	41/64 (64.1%)
		Not seen	16/64 (25.0%)
		Decreased	6/64 (9.4%)
		Increased	1/64 (1.6%)

Please assess and provide comment on the percentage of plasma cells within the aspirate

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	0% – 5%	0% – 5%	64/64 (100.0%)

Please provide any other sample comments following assessment of the aspirate:

A brief summary of the comments provided:

- Normocellular to slightly raised cellularity for a 2-year-old
- Several normocellular bone particles and trails for patient's age
- Severe trilineage dysplasia
- Increased blasts
- No plasma cells observed and few megakaryocytes
- Dysplastic features particularly in the myeloid series
- Hypogranular neutrophils
- Immature monocytoid cells present
- Increased monoblasts and myeloblasts
- Increased macrophage activity with excess pigment
- Pseudo-Gaucher cells
- Increased haematogones
- Prominent eosinophilia and basophilia

Should flow cytometry testing be undertaken following this bone marrow aspirate morphology assessment?

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Yes	Yes	60/64 (93.8%)
		No	4/64 (6.3%)

What flow cytometry testing do you think should be undertaken following bone marrow aspirate morphology assessment?

Your Response(s)	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Screen for acute leukaemia	Screen for acute leukaemia	55/64 (85.9%)
		N/A	5/64 (7.8%)
		Screen for acute leukaemia / Screen for lymphoproliferative disorder	3/64 (4.7%)
		Screen for lymphoproliferative disorder	1/64 (1.6%)

Are there any other laboratory tests that you think should be undertaken after the bone marrow aspirate assessment?

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
	Yes	Yes	63/64 (98.4%)
		No	1/64 (1.6%)

Please see below a table showing the further testing requested by participants –

Your response -

Test	Number of Returns
Cytogenetics	42
Myeloid gene panel	38
BM trephine/biopsy	28
Karyotype	25
Molecular genetics	14
NGS	14
FISH	9
FBC	7

Test	Number of Returns
SNP Array (if Karyotype fails)	5
Iron stain	3
B12 and Folate	2
Immunostaining	2
LDH	2
FBC Tumour lysis investigations	2
Coagulation profile	1
Crossmatch	1

Report Commentary

The first stage of the exercise was the identification of specific cells within the bone marrow aspirate smear. For this exercise, 100% agreement was not reached for any cell. Cell A was identified as a Pelger–Huët cell by 60.9% of participants. This cell was also identified as a neutrophil by 35.9% of participants. A Pelger–Huët cell is an anomaly of neutrophils [1] indicating a 96.8% agreement that the cell is a neutrophil. Cell B was identified as a Nucleated Red Blood Cell (All stages except Proerythroblast) by 54.7% of participants with 35.9% classifying the cell as a Proerythroblast showing 90.6% agreement as to the lineage of the cell. No consensus was reached for Cell C however 75% of participants classified the cell as within the myeloid series. Cell D was identified as a neutrophil by 59.4% of participants. Cell E was identified as a large granular lymphocyte by 73.4% of participants. One participant reported 'none of the above' for cells A to E as they were unable to locate the letters to identify the cells.

This exercise was the first using our new slide hosting service, Precipoint. As expected with a totally new software there were a few slight issues with participants unable to locate the 5 pre-labelled cells for identification. To ensure that this issue is addressed for future exercises, please find the instructions included on page 10 of this report.

Please see below the link for website instructions –

http://www.ukneqasli.co.uk/app/download/5817176923/Participant+Instructions+for+Viewing+Precipoint+Scanned+Images.pdf

Increased cellularity was reported by 51.6% of participants. Megakaryopoiesis, erythropoiesis and myelopoiesis were each classified by participants as decreased (78.1%), normal (59.4%), and increased (59.4%) respectively. The presence of dysplasia was identified in both the erythroid lineage (53.1% of participants) and myeloid lineage (54.7% of participants) at between 10% and 50% but no consensus was reached for the presence of dysplasia in the megakaryocytic lineage.

Finally, the third stage of the exercise asked participants what further testing, if any, should be undertaken. Immunophenotyping for acute leukaemia was suggested by 58/64 participants. Within this group,3 participants stated that additional screening for a lymphoproliferative disorder should also be carried out. One participant stated immunophenotyping for a lymphoproliferative disorder alone would suffice and 5 participants stated that immunophenotyping was not required at all.

In addition to immunophenotyping by flow cytometry, further testing was felt to be necessary by 98.4% of participants with the recommended testing centring around cytogenetics, myeloid gene panel and BM trephine/biopsy. One participant stated that no further tests would be required but then stated that they would request FISH, Karyotype and Myeloid gene panel.

Potential diagnoses submitted for this exercise included Fanconi Anaemia related MDS in accelerated phase - EB-2, MDS-EB1, Acute myeloid leukaemia, Acute myelomonocytic leukaemia, Double pathology CMML1 plus a low-grade lymphoproliferative disorder, MDS EB2/CMML2 plus a low-grade lymphoproliferative disorder.

UK NEQASLI would like to thank participants for completing the survey included in the HMBMAA 212204 exercise. This information has provided useful comments and suggestions on how the programme can be improved to better reflect practices in hospital laboratories. Where possible, changes will be incorporated into the programme over future exercises.

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

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, Fax: +44 (0) 114 267 3601
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/

4.8.4) The UK NEQAS LI Policy for the Use of Reports by Individuals and Organisations states that all EQA reports are subject to copyright, and, as such, permission must be sought from UK NEQAS LI for the use of any data and/or reports in any media prior to use. See associated policy on the UK NEQAS LI website: http://www.ukneqasli.co.uk/eqa-pt-programmes/new-participant-information/