West Herts Teaching Hospitals NHS Trust operating
UK NEQAS Haematology and Transfusion

Overall Summary

Specimen 2307BF1	
Age (years)	79
Sex	M
Hb(g/L)	98
WBC (10^9/L)	15.6

Specimen 2307BF2	
Age (years)	79
Sex	F
Hb(g/L)	102
WBC (10^9/L)	142.1

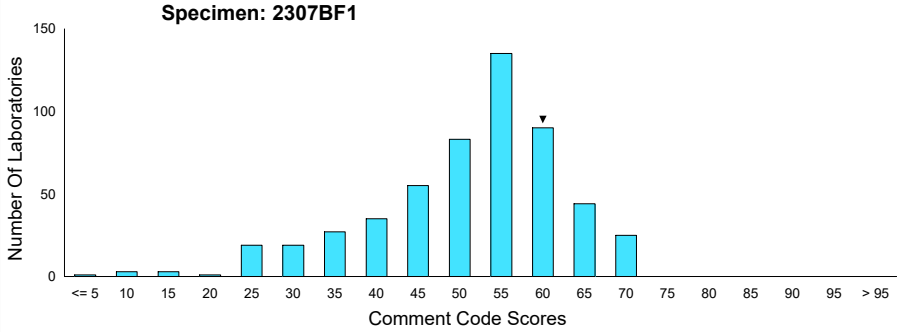
Specimens were distributed to 565 participants.
542 participants returned results.
This represents a 95% return rate.

Non Participation Score: 0

Specimen Quality	**2307BF1**	**2307BF2**
Satisfactory	536	539
Unsatisfactory	6	3
You reported:	Satisfactory	Satisfactory

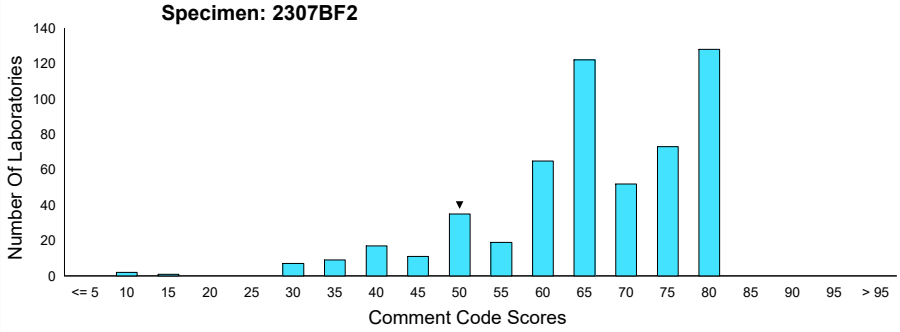
Scoring Information

Specimen: 2307BF1



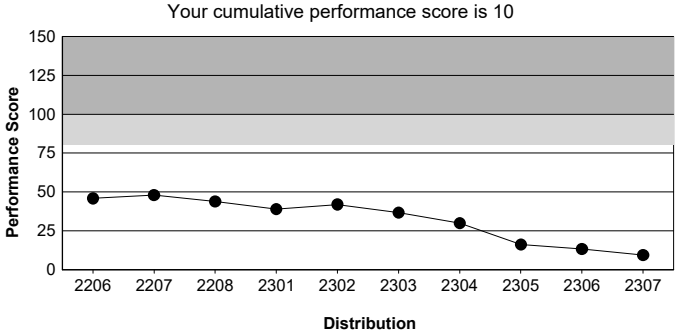
Your Result	**59**
Target Value ≥	**52**
DI	0
Your flags	**A / a**
Expert flags	
Lymphocytosis : A	
Abnormal/Suspect Neoplastic Lymphocytes : a	

Specimen: 2307BF2



Your Result	**48**
Target Value ≥	**64**
DI	1.5
Your flags	**A / X**
Expert flags	
Blast Cells : A	
Nucleated RBCs : a	

Blood Films for Morphology
Your cumulative performance score is 10



Your Comments:

For information on data analysis and performance assessment see the UK NEQAS Haematology Participants' Manual (www.ukneqash.org)
Scheme Director: Barbara De la Salle
UK NEQAS (H), PO Box 14, WATFORD WD18 0FJ, UK
email:haem@ukneqas.org.uk Phone: +44 (0) 1923 587 111
Authorised by: Yvonne Hector (Service Manager)

UKAS PROFICIENCY TESTING
7805

Data Analysis

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

Code	Comment	Rank	Number	Score
227	Lymphocytosis	1	502	22
302	Thrombocytopenia	2	418	19
017	Rouleaux	3	250	11
229	Abnormal/Suspect Neoplastic Lymphocy	4	239	11
219	Atypical/Suspect Reactive Lymphocytes	5	110	5
211	Hypogranular/Agranular Cytoplasm (Neu	6	77	3
218	Smear/Smudge Cells	7	73	3
004	Hypochromic Cells	8	69	3
002	Microcytes	9	59	3
007	Poikilocytes	10	54	2

Your Results

Your reported comments with the number who reported that comment

Code	Comment	Rank	Number	Score
227	Lymphocytosis	1	502	22
302	Thrombocytopenia	2	418	19
229	Abnormal/Suspect Neoplastic Lymphocy	4	239	11
219	Atypical/Suspect Reactive Lymphocytes	5	110	5
214	Prolymphocytes	10	54	2

Your 'Other morphology'

002 - Microcytes; 016 - Spherocytes

Your suggested diagnosis

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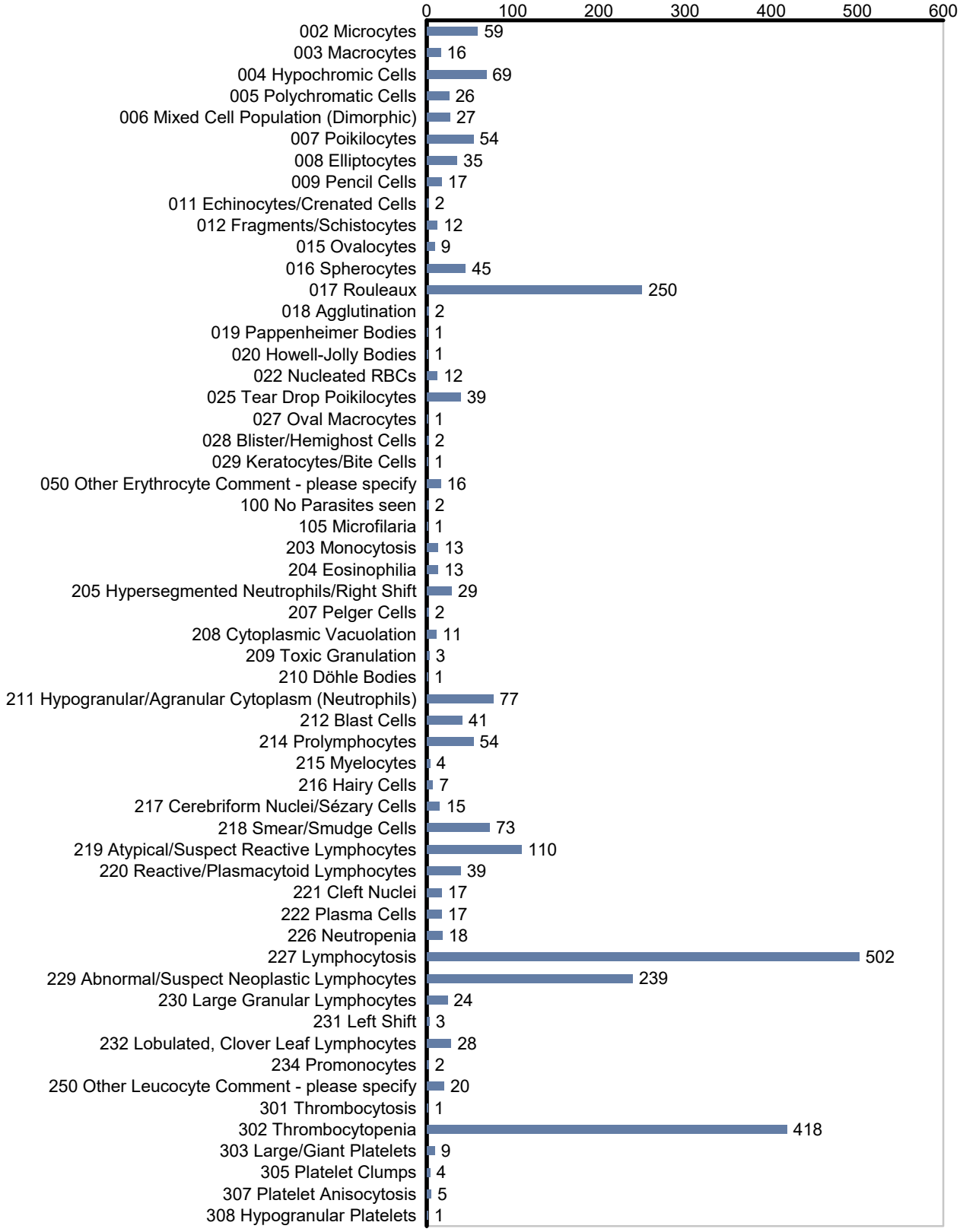
Analysis of Morphology Codes reported by Participants



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Data Analysis

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

Code	Comment	Rank	Number	Score
022	Nucleated RBCs	1	499	21
212	Blast Cells	2	439	19
302	Thrombocytopenia	3	398	17
218	Smear/Smudge Cells	4	325	14
208	Cytoplasmic Vacuolation	5	174	7
011	Echinocytes/Crenated Cells	6	172	7
227	Lymphocytosis	7	168	7
229	Abnormal/Suspect Neoplastic Lymphocy	8	126	5
005	Polychromatic Cells	9	40	2
024	Acanthocytes	10	28	0

Your Results

Your reported comments with the number who reported that comment

Code	Comment	Rank	Number	Score
212	Blast Cells	2	439	19
302	Thrombocytopenia	3	398	17
011	Echinocytes/Crenated Cells	6	172	7
229	Abnormal/Suspect Neoplastic Lymphocy	8	126	5
215	Myelocytes	14	20	0

Your 'Other morphology'

022 Nucleated RBCs

Your suggested diagnosis

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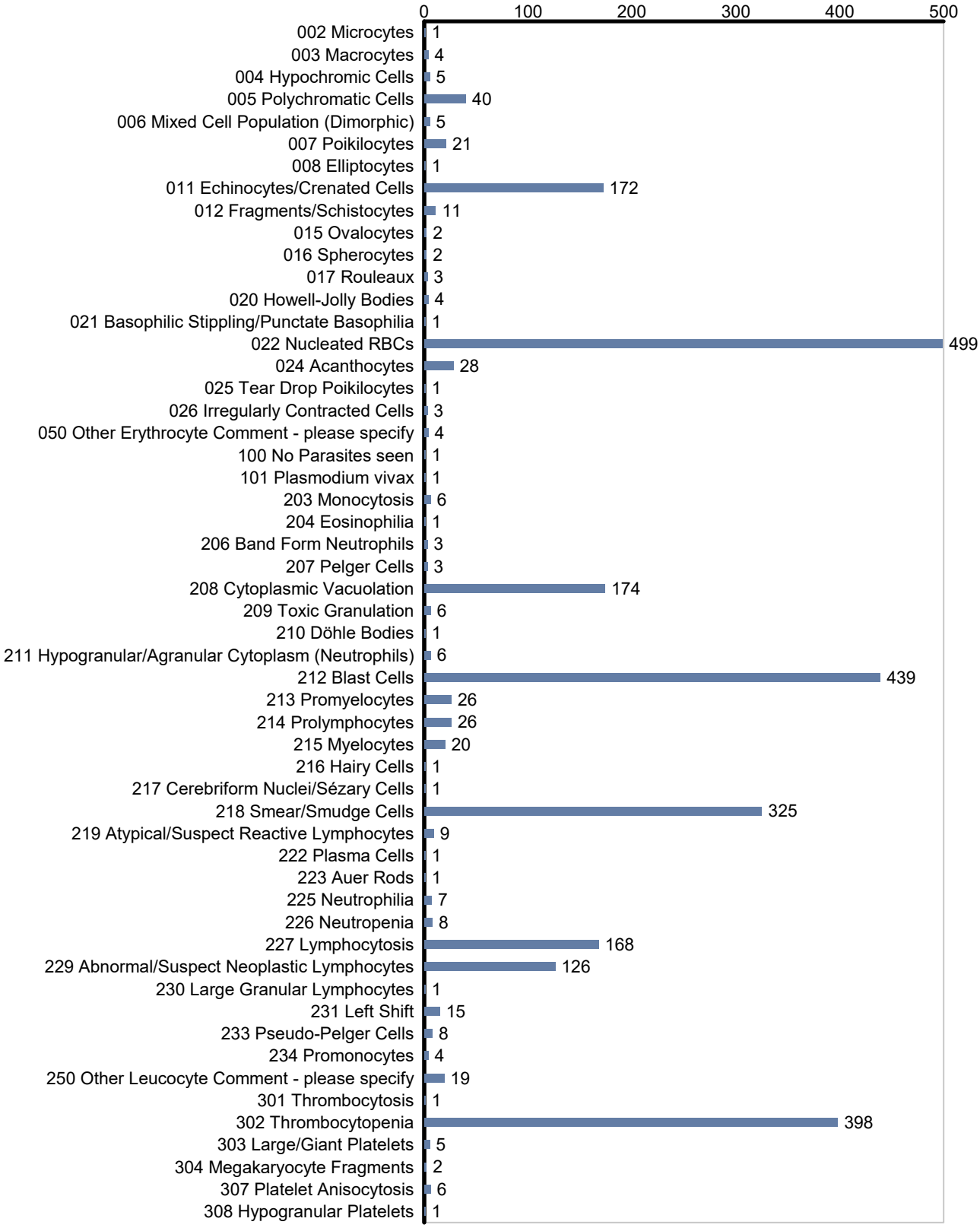
Analysis of Morphology Codes reported by Participants



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Film 2307BF1 – Clinical Details

This film was from a 79-year-old male

Full Blood Count results, including automated differential count, for 2307BF1

Parameter	Result	Normal Range	Parameter	Result (x10^9/L)	Normal Range
RBC (10^{12}/L)	4.02	4.40 – 5.80	**WBC**	15.6	3.0 – 10.0
Hb (g/L)	98	130 – 170	Neutrophils	4.1	2.0 – 7.5
Hct (L/L)	0.32	0.37 – 0.50	Lymphocytes	10.1	1.5 – 4.0
MCV (fL)	78.6	80.0 – 99.0	Monocytes	1.0	0.2 – 1.0
MCH (pg)	24.3	26.0 – 33.5	Eosinophils	0.3	0.0 – 0.4
MCHC (g/L)	309	300 – 350	Basophils	0.0	0.0 – 0.1
RDW (%)	20.8	11.5 – 15.0	NRBC	0.0	0.0 – 0.0
Platelets (10^9/L)	93	150 – 400			

Further results:

Biochemistry

Parameter	Result	Normal Range	Parameter	Result	Normal Range
Sodium (mmol/L)	142	133 – 146	Alanine Transaminase (U/L)	33	0 - 52
Potassium (mmol/L)	4.2	3.5 – 5.3	Alkaline Phosphatase (U/L)	108	30 - 160
Urea (mmol/L)	5.7	2.5 – 7.8	Albumin (g/L)	42	35 – 50
Creatinine (µmol/L)	87	60 – 106	Calcium (mmol/L)	2.47	2.20 – 2.60
Bilirubin (µmol/L)	9	0 - 21	Adjusted Calcium (mmol/L)	2.43	2.20 – 2.60
Phosphate (mmol/L)	1.03	0.8- 1.50	LDH (U/L)	263	0 - 250
Ferritin (µg/L)	134	30 - 400	Vitamin B12 (ng/L)	513	180 - 999
Folate (µg/L)	7.0	>3			

Immunophenotyping

B cells (CD19+) account for 42.97% of total nucleated cells (TNCs) with a Kappa/Lambda ratio of 0.001:1, with phenotype CD19+, CD5 heterogeneous, CD10-, CD20+, CD23+, CD79b weak, CD200+, CD81 weak, CD43 weak, ROR 1 heterogeneous, weak SmIg (Lambda). Hairy cell markers negative.
T cells (CD3+) account for 20.60% of TNCs with a CD4/CD8 ratio of 3.25:1. NK cells account for 1.63% of TNCs.
Summary: Clonal CD19+, CD5 heterogenous, CD10- B cell population identified.

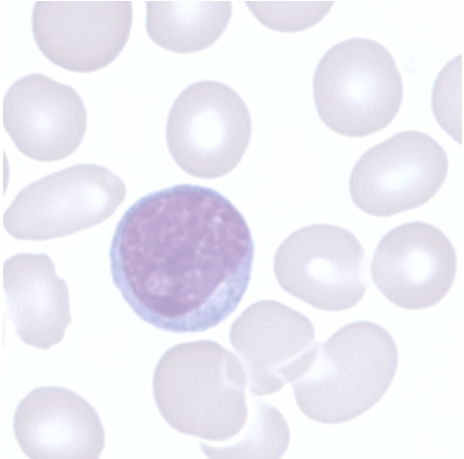

Figure 1

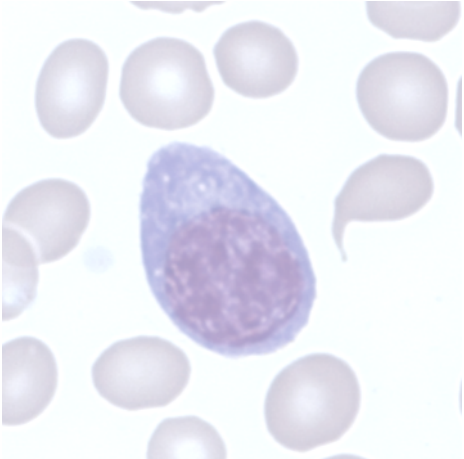

Figure 2

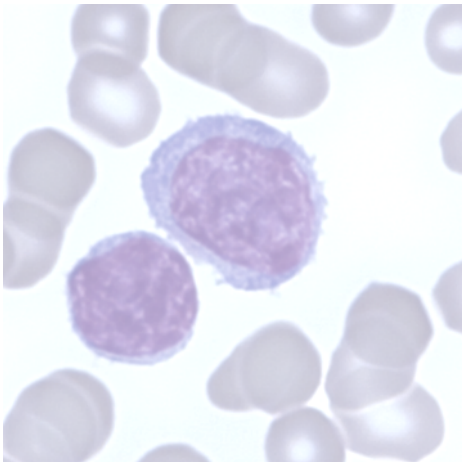

Figure 3

Expert comment *(Dr Chris McNamara)*

This film was made from a 79-year-old man with an established history of chronic lymphocytic leukaemia (CLL). No other information was available e.g. whether this man had received treatment or whether he was well or unwell. The vast majority (93%) of participants noted the lymphocytosis, a requirement for a diagnosis of CLL and most respondents suggested this diagnosis. The incidental finding of a mild lymphocytosis is a very common event in people having a full blood count for various reasons because many people with CLL are asymptomatic for long periods of time before suggestive symptoms develop.

A minority of respondents suggested alternative lymphoproliferative diagnoses e.g. mantle cell lymphoma (MCL) and hairy cell leukaemia (HCL). Note the occasional, small villous projections (depicted in figure 3), seen in a minority of the lymphocytes. Differentiation between CLL and hairy cell leukaemia can usually be made using morphology e.g. in HCL the nucleus is often indented or kidney-shaped and the majority of the lymphocytes will have prominent circumferential projections, rather than a few cells showing minor projections, which may be secondary to post-analytical preparation changes in the laboratory. For a discussion on differentiation between CLL and MCL see the next case (2307BF2). However, integrating morphologic findings with flow cytometry adds to the accuracy of the diagnosis. The immunophenotype reported here is entirely typical of CLL. Note that over-expression of CD200 is widely reported in CLL but not in mantle cell lymphoma, aiding discrimination between these two CD5-positive B-cell malignancies. Molecular studies could also be helpful but take considerably longer than immunophenotyping.

Forty-six per cent of respondents commented on the presence of red cell rouleaux, which refers to the arrangement of red cells adjacent to one another in such a way that they resemble a stack of coins. Causes of rouleaux in people affected by CLL include the presence of an abnormal monoclonal paraprotein (this is then termed a 'monoclonal gammopathy') which occurs in a minority of people with this condition. Other causes include infection and inflammation, or any other condition associated with the presence of proteins in the plasma of high molecular weight. Many respondents suggested synchronous infection to account for the rouleaux. As already mentioned, it is important to recall that people with CLL typically have a long disease course, now often spanning several decades and are at risk of developing other unrelated disease processes, so infection is an important consideration for these changes. Some respondents suggested the possibility of plasma cell myeloma to account for the film findings. Although rouleaux is commonly seen in myeloma the morphological features are those of lymphocytes and not plasma cells. No information was available in this man's case as to the presence of an abnormal protein in the serum.

Thrombocytopenia was reported by 77% of respondents. This is a common finding in CLL and can be due to one or more of the following: reduced production of platelets in the bone marrow, due to replacement of normal haematopoiesis by CLL, immune-mediated destruction of platelets as a consequence of immune dysregulation secondary to CLL and, finally, sequestration in the spleen in people who have splenomegaly due to infiltration of the spleen by their disease.

In summary, most respondents accurately reported the mild lymphocytosis and thrombocytopenia, and many suggested the correct diagnosis of CLL and offered the pathway their lab would follow to arrive at this diagnosis. A range of other blood film findings may be detected in CLL and it is helpful to be aware of these as blood films from people with this condition are commonly encountered in the haematology laboratory.

Film 2307BF2 – Clinical details

This film was from a 79 year old female

Full Blood Count results, including automated differential count, for 2307BF2

Parameter	Result	Normal Range	Parameter	Result (x10^9/L)	Normal Range
RBC (10^{12}/L)	3.26	4.40 – 5.80	**WBC**	140.0	3.0 – 10.0
Hb (g/L)	102	130 - 170	Neutrophils	26.9	2.0 – 7.5
Hct (L/L)	0.30	0.37 – 0.50	Lymphocytes	20.2	1.5 – 4.0
MCV (fL)	91.7	80.0 – 99.0	Monocytes	92.7	0.2 – 1.0
MCH (pg)	31.3	26.0 – 33.5	Eosinophils	0.0	0.0 – 0.4
MCHC (g/L)	341	300 – 350	Basophils	0.3	0.0 – 0.1
RDW (%)	18.6	11.5 – 15.0	NRBC	2.1	0.0 – 0.0
Platelets (10^9/L)	97	150 – 400			

Further results:

Biochemistry

Parameter	Result	Normal Range	Parameter	Result	Normal Range
Sodium (mmol/L)	145	136 – 145	Bilirubin (µmol/L)	19	<21
Potassium (mmol/L)	4.5	3.5 - 5.1	Globulin (g/L)	9	18 – 36
Urea (mmol/L)	12.3	2.8 – 8.1	Alanine Transaminase (U/L)	12	<33
Creatinine (µmol/L)	209	44 – 80	Alkaline Phosphatase (U/L)	101	35 – 104
C-Reactive Protein (mg/L)	50	0 – 5	Albumin (g/L)	26	35 – 52
Creatine Kinase (u/L)	62	<170	Calcium (mmol/L)	2.13	2.15 – 2.60
Phosphate (mmol/L)	1.16	0.81 – 1.45	Adjusted Calcium (mmol/L)	2.45	2.15 – 2.60
Chloride (mmol/L)	110	95 – 105	Total Protein (g/L)	35	60 – 80
Magnesium (mmol/L)	1.03	0.66 – 0.99			

Immunophenotyping
Results from a lymphoid gate which accounts for 92.50% of TNCs.
B cells (CD19+) account for 88.11% of TNCs with a Kappa/Lambda ratio of 10631.25:1 with phenotype CD19+, CD5+, CD10-, CD20+ bright, CD23-, CD79b+, CD200-, CD81 weak, CD43-, ROR 1+, CD123+ weak heterogenous, CD25-, CD103-, CD11c-, bright SmIg (Kappa).
T cells (CD3+) account for 3.21% of TNCs with a CD4/CD8 ratio of 0.77:1.
NK cells account for 0.75% of TNCs.
Summary: Clonal CD19+, CD5+, CD10- B-cell population identified.

FISH analysis
Interphase FISH analysis detected the presence of an *IGH::MYEOV*, *CCND1* [t(11;14)] rearrangement in 120 out of 150 cells, consistent with the t(11;14) detected by G-banded chromosome analysis previously) IOG 6082773, received 14/05/2015).
No evidence of a *TP53* deletion.
Interphase FISH analysis of 150 cells showed the presence of a normal signal pattern for the *TP53* (17p13) locus.
These findings are consistent with relapsed mantle cell lymphoma.

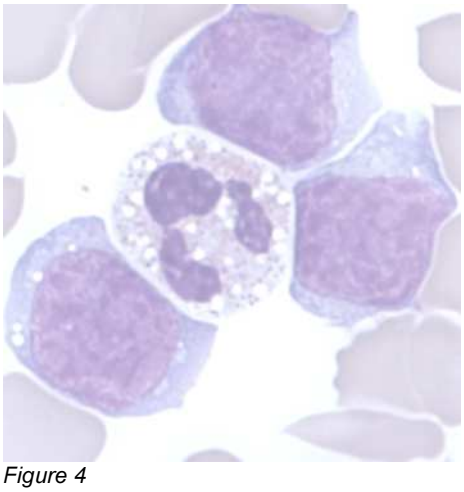
Figure 4

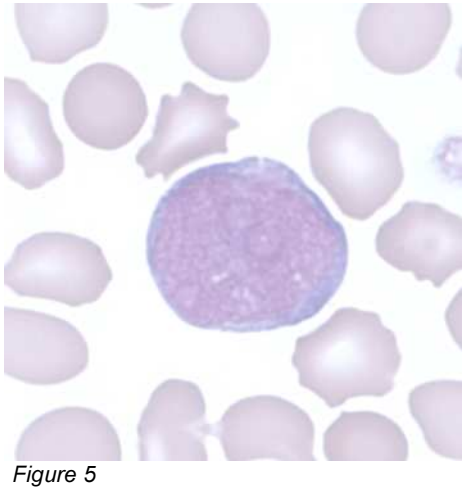
Figure 5

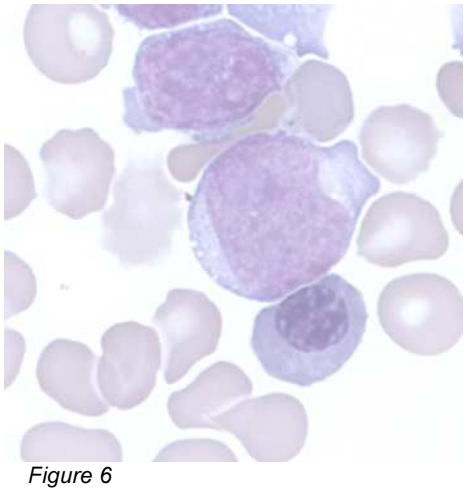
Figure 6

Expert comment *(Dr Chris McNamara)*

This film was prepared from a 79-year-old woman with an established history of mantle cell lymphoma (MCL). No other information was available, e.g. whether she had ever received treatment, whether she was well or unwell at the time the blood film was made. Involvement of the peripheral blood by MCL is very common. The majority (81%) of respondents noted the presence of large, abnormal mononuclear cells and 60% reported smear cells in the preparation. Ninety-two percent reported the presence of nucleated red blood cells and/or leukoerythroblastosis. In the absence of any clinical background being provided, participants proposed a range of potential explanations to account for these findings. An important point to consider in approaching this case is that MCL is comprised of a range of

morphological variants, some of which have clinical significance and are important for laboratories to be able to recognise. The appearances of MCL in the blood may mimic the more commonly encountered chronic lymphocytic leukaemia (CLL-see also case 2307BF1), prolymphocytic leukaemia and even acute lymphoblastic leukaemia. All of these were suggested by some respondents. Although there were some occasional, small lymphocytes with mature chromatin and indistinct nucleoli, the presence of the immature, highly pleomorphic cells like those seen in figures 4, 5 and 6, coupled with the leukoerythroblastic picture make the diagnosis of uncomplicated CLL very unlikely.

The key to differentiating between the other possibilities is clinical correlation (which in this case would have elicited the background of MCL) followed by interrogation of the lymphocytes using immunophenotyping and molecular studies. The phenotype reported above is typical of MCL and allows for differentiation from CLL; note the strong expression of surface immunoglobulin and the absence of both CD23 and CD200 expression. Fluorescence *in situ* hybridisation (FISH) showed the archetypal MCL translocation that is thought to be the primary initiating event in this condition. This translocation involves an immunoglobulin promoter and a gene encoding for the protein called cyclin D1. This latter protein is able to overcome the suppression of the cell cycle by other cellular proteins resulting in uncontrolled cellular proliferation. Interestingly, this episode of relapse was not associated with any change in the *TP53* gene. Alterations in this gene have recently been recognised to be highly prognostic in the relapsed MCL setting. The morphologic appearances in this case are typical of the blastoid or pleomorphic variant of MCL, both of which tend to have a more aggressive clinical course. Note that people with a diagnosis of MCL may present with the classical form of MCL and relapse with the blastoid form, and *vice versa*.

The causes of thrombocytopenia (reported by 72% of respondents) in this setting are variegate and include reduced production of platelets due to replacement of the marrow by MCL, immune-mediated destruction of platelets due to immune dysregulation caused by MCL and sequestration of platelets in an enlarged spleen. This degree of thrombocytopenia may also be unrelated to MCL e.g., infection, inflammation and medications. The presence of toxic changes in the film (see the cytoplasmic vacuolation, reported by 32% of respondents, in a neutrophil depicted in figure 4) raised the possibility of a concomitant infectious or inflammatory process. I am grateful for the observation that this case demonstrated numerous pseudoplatelets which are fragments of the abnormal leukaemic cells which may be erroneously counted as platelets due to their similar size. The actual platelet count may be lower than that estimated by the laboratory analyser unless an immunological method is employed to enumerate the platelets. If this function is not available, then it would be prudent for laboratories to calculate the ratio of platelets to the white cells to estimate the true platelet count.

In summary, this blood film represents a relapse of known MCL and the morphological appearances are highly suggestive of the blastoid or pleomorphic variant of the condition. The blood film findings were well documented by the majority of respondents. The case well demonstrates the necessity of integrating morphologic findings with other modalities for a rapid and accurate diagnosis.

Scoring update (Barbara De la Salle)

Participants will be aware that we have developed a scoring system for Blood Film comments, based on your agreement with the consensus of returns by all participants and the significant features identified by our expert commentary panel. We have provided a notice giving more detail of the system with this report. Scoring remains on a 'shadow' basis at the time of writing and is supplied for your information only. If you have any queries or comments, please forward them to haem@ukneqas.org.uk.