



BBTS Annual
Conference 2016

UK NEQAS Low Level Leucocyte Enumeration Programme

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UK NEQAS for Leucocyte Immunophenotyping

Content

- Results of the UKNEQASLI flow cytometry survey
- Analysis of UKNEQASLI EQA material using FACSVia
- Low Level Leucocyte Report
- Further Improvements

How many low level leucocyte counts do you perform per month?

Tests per month	Number of laboratories (n=56)
0 to 25	18
26-50	19
51-75	2
76-100	1
101-125	0
126-150	1
151-175	1
176-200	1
201-225	1
226-250	1
251-275	2
401-500	3
>501	6

What percentage of products are tested for an absolute Low level Leucocyte count?

*2 centres stated 2 sampling rates, of 1% for RBCs and 100% for platelets. RBC data used

**1 centre stated 2 sampling rates, of 5% for RBCs and 100% for platelets. RBC data used

No UK Consensus despite guidelines

Percentage of products tested	Number of laboratories (n=30)
1*	14 (47%)
1.5	1 (3%)
2.5	1 (3%)
5**	5 (17%)
6	1 (3%)
7	1 (3%)
10	3 (10%)
50	1 (3%)
80	1 (3%)
100	2 (7%)

What is the minimum absolute Low Level Leucocyte count where a unit would be rejected?

*1 centre stated 2 rejection rates, of 1×10^5 per unit for RBCs and 2×10^5 per unit for platelets. RBC data used

No UK Consensus despite guidelines

Limits for Rejection	Number of laboratories (n=24)
>0.5 cells/ μ l	1
>1 cell/ μ l	1
>3 cells/ μ l	1
>5 cells/ μ l	1
>20 cells/ μ l	1
> 1×10^5 per unit*	3
> 1×10^6 per unit	8
> 2×10^5 per unit	1
> 5×10^5 per unit	1
> 5×10^6 per unit	6

Is this number decided in-house or is it in line
with national guidelines?

In-House	18/64 (28%)
National Guidelines	46/64 (72%)

If guidelines were used, please provide the reference.

Brazilian Ministry of Health guide

BSQR Blood Transfusion Service Guidelines (Red Book)

Council of Europe - Recommendation R (95) 15

Developed in house using national guidelines and QC testing principles.

EU Blood Directive 2204/33/EC

Guide to preparation, use and quality assurance of blood components-recommendation n.(R)9515-16th ed)

Guide to the preparation, use and quality assurance of blood components 9° edition

Guidelines for Blood Transfusion in UK 2005

Standards of Practice for Blood Transfusion

Statistical process control

Unknown - handled by Blood bank

Which software do you use to analyse your results?

Software Used	Number of laboratories (n=44)
Cellquest (Pro)	11
CXP Software	10
Diva	13
Navios Software	4
Kaluza	1
Multiset	1
Excel	3
Expo32	1

Analysis of EQA material on the FACSVia

ID: 557b

Report Date: OCT 23, 2015 13:58:28 PDT

BD FACSVia Serial #: AC65687410038
BD FACSVia Software Version: 1.0

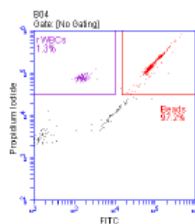
Name: PLT NEQAS

Case #:

Date Acquired	OCT 23, 2015 12:50:59 PDT	Operator
Trucount Lot #	15121	Prep
Trucount Bead Count	47150	Tube Rack
Trucount Expiration Date	MAY 31, 2017	Lab Director
Pack Volume in mL		

BD FACSVia File Name: C:\Users\Admin\Documents\NEQAS 231015.cs

Leucocount



	Event Count	Abs r WBCs/ μ L	Abs r WBCs/pack
Beads	10000		
r WBCs	132	6.2	

QC Messages

Comments:

ID: 557b

Report Date: OCT 23, 2015 13:52:42 PDT

BD FACSVia Serial #: AC65687410038
BD FACSVia Software Version: 1.0

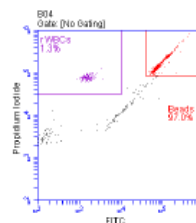
Name: PLT NEQAS

Case #:

Date Acquired	OCT 23, 2015 12:50:59 PDT	Operator
Trucount Lot #	15121	Prep
Trucount Bead Count	47150	Tube Rack
Trucount Expiration Date	MAY 31, 2017	Lab Director
Pack Volume in mL		

BD FACSVia File Name: C:\Users\Admin\Documents\NEQAS 231015.cs

Leucocount



	Event Count	Abs r WBCs/ μ L	Abs r WBCs/pack
Beads	9982		
r WBCs	132	6.2	

QC Messages

Comments:

Analysis of EQA material on the FACSVia

RBC NEQAS	Default gate (cells/ μ l)	Regated (cells/ μ l)
553a	9.6	9.7
553b	8.5	8.5
554a	5	5
554b	4.2	4.3
555a	20.4	20.5
555b	20.6	20.7
556a	1.8	1.9
556b	1.7	1.7
557a	2.8	2.8
557b	2.8	2.8
558a	1.7	1.7
558b	1.7	1.7

Analysis of EQA material on the FACSVia

PLT NEQAS	Default gate (cells/ μ l)	Regated (cells/ μ l)
553a	17.7	18.1
553b	18.1	18.5
554a	6	6.2
554b	5.4	5.5
555a	8.3	8.4
555b	7.9	8
556a	0.7	0.7
556b	0.8	0.8
557a	5.6	5.6
557b	6.2	6.2
558a	*	*
558b	2.6	2.6

* Run in singlicate

Comparison of FACSVia data to trial data

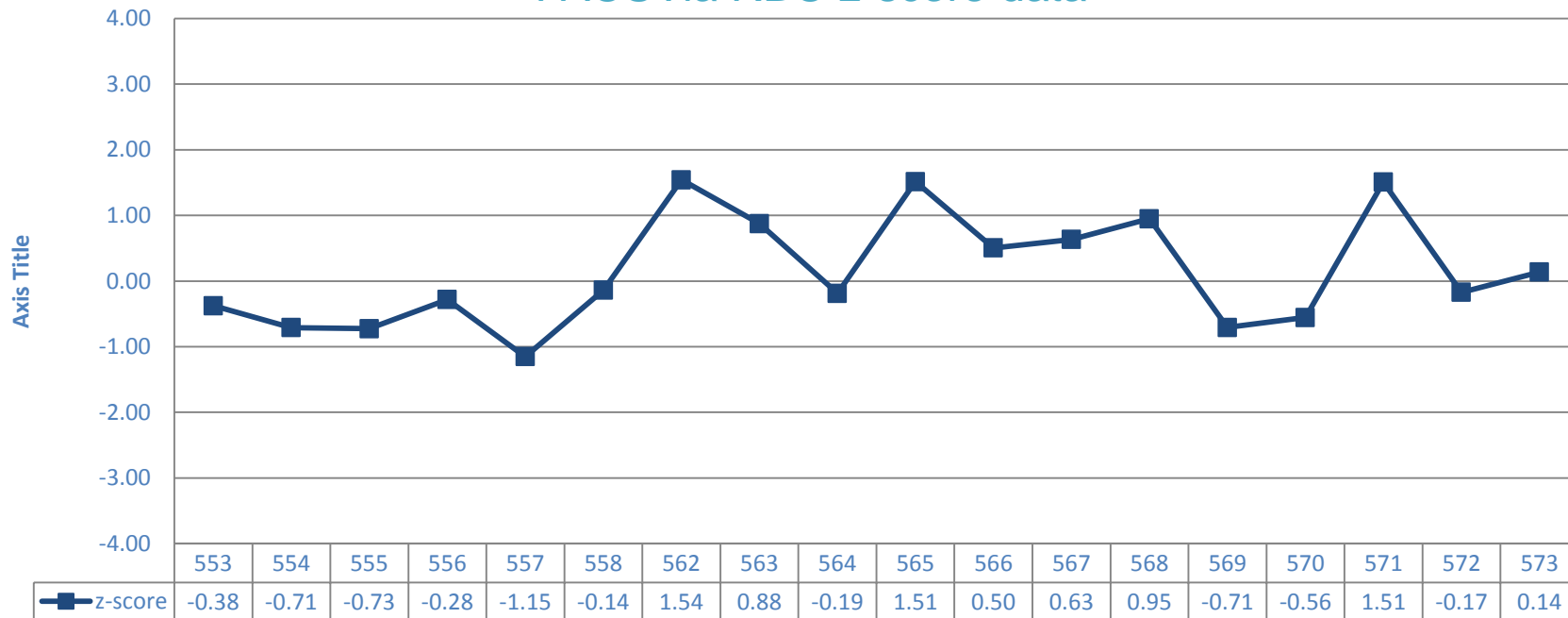
Sample	Result (cells/ μ l)	Robust Mean	Robust SD	z-score	Classification
RBC 553	9.05	9.57	1.38	-0.37681	Satisfactory
RBC 554	4.6	5.16	0.79	-0.70886	Satisfactory
RBC 555	20.5	22.61	2.91	-0.72509	Satisfactory
RBC 556	1.75	1.84	0.32	-0.28125	Satisfactory
RBC 557	2.8	3.41	0.53	-1.15094	Satisfactory
RBC 558	1.7	1.75	0.36	-0.13889	Satisfactory

Comparison of FACSVia data to trial data

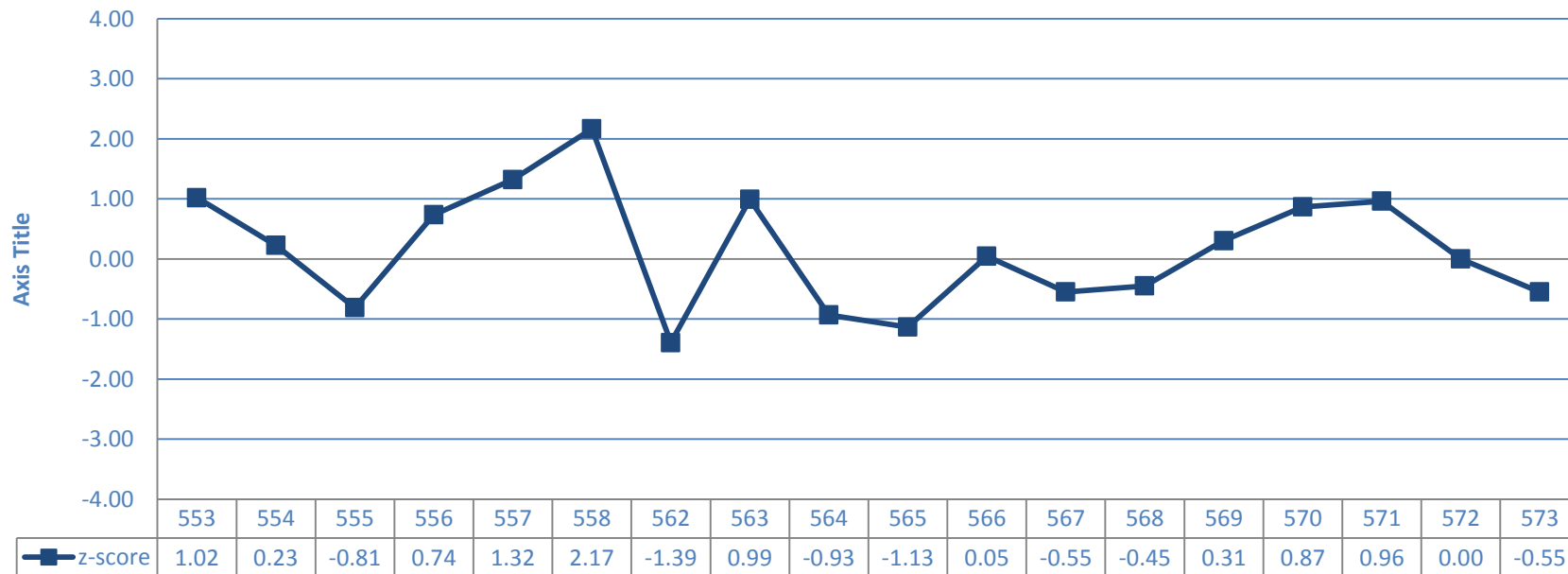
Sample	Result (cells/ μ l)	Robust Mean	Robust SD	z-score	Classification
PLT 553	17.9	16.41	1.46	1.02	Satisfactory
PLT 554	5.7	5.53	0.75	0.23	Satisfactory
PLT 555	8.1	8.66	0.69	-0.81	Satisfactory
PLT 556	0.75	0.61	0.19	0.74	Satisfactory
PLT 557	5.9	5.12	0.59	1.32	Satisfactory
PLT 558	2.6	1.82	0.36	2.17	Action *

* Sample run in singlicate

FACSVia RBC z-score data



FACSVia PLT z-score data



Changes to the format of LLL reports and performance monitoring

Performance monitoring

- Review carried out by Statistical Services Unit of University of Sheffield - September 2012
- Concluded that
 - Systems were fit for purpose
 - Unsatisfactory performing laboratories were genuinely outside of consensus values
 - Systems met ISO 17043 requirements
- So why change?



The Royal College of Pathologists

Pathology: the science behind the cure

**Review of cellular pathology governance,
breast reporting and immunohistochemistry at
Sherwood Forest Hospitals NHS Foundation Trust**

**A report prepared for the Care Quality Commission
in respect of diagnostic and screening procedure**

20 February 2013

- Section 3.7.4 (abridged) Outlined Essential EQA Scheme Criteria
- Timeliness in identifying problems
- Targeting of clinical decision points.
- Identification of persistent poor performance: schemes should be designed with the purpose of ensuring patient safety as their primary aim.
- Consistency in identification of persistent poor performance
- Benchmarking: laboratories should be provided with feedback allowing them to benchmark their performance against past performance and that of other participants.
- Transparency

2013/2014 Scoring Review

- 20 programmes with 10 different scoring systems
 - Mix of quantitative and qualitative statistics
- Quantitative Programmes
 - Immune Monitoring, CD34, **Low Level Leucocyte**, Leukaemia Immunophenotyping, Minimal Residual Disease, Chimerism, BCR-ABL Quantitation
- Qualitative Programmes
 - PNH, PNH High Resolution, 11 other Molecular Programmes

Use of z-scores

- z-scores involve the calculation of a robust average and SD (ISO 13528 provides the algorithm)
- $\text{z-score} = [\text{lab value} - \text{robust average}] / \text{robust SD}$
 - Range of -2 to +2 is acceptable
 - <-2 to -3 and >2 to 3 are 'actions'
 - <-3 and >3 are 'critical'
 - 2 'actions' in a 3 sample window = 'critical'

Performance Monitoring-Old Format

for Leucocyte Immunophenotyping

Low Level Leucocyte Counting Scheme

Sheffield Teaching Hospitals NHS Foundation Trust

Trial No: 131408

Participant: [Redacted]

Date Issued: 28-January-2014

Date Closed: 14-February-2014

Machine 1: machine 1

Machine 2: [Redacted]

Negative: False

Your Results **Sample:** RBC513

Machine	Result	Score	Running Score (Last 5 Samples)	Overall Performance
machine 1	6.800	5	30	Satisfactory

Performance Graphs

machine 1

Centile Range

5%	10%	25%	Median	75%	90%	95%
4.060	5.876	7.030	7.900	8.660	9.352	9.516

Calculated Absolute Leucocyte Count RBC513

Your Results

Machine 1 : 6.80

Machine 2 : No Result

Negative : No Result

Issue Date: 17 February 2014

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Method Breakdown for Calculated Absolute Count (All Results)

RBC513

All Methods	No. In Group	Lower Quartile	Median	Upper Quartile	CV%
Flow Cytometry	97	7.03	7.90	8.66	20.63

Flow Cytometry Sub-Group Analysis	No. In Group	Lower Quartile	Median	Upper Quartile	CV%
BD-Facscalibur & BD Leucocount	37	7.03	7.68	8.30	16.54
Coulter-Navios & BD Leucocount	6	8.51	8.59	9.08	6.66
Coulter-Navios & Leukosure	3	6.34	7.98	8.14	23.81
Coulter-XL & BD Leucocount	3	7.34	7.81	8.81	18.82
Coulter-FC-500 & Coulter DNA-Prep	3	8.62	9.15	9.51	5.01
Coulter-FC-500 & Leukosure	5	4.20	6.30	8.00	50.32
BD-Facs Canto & BD Leucocount	4	6.78	7.15	7.30	7.31
BD-Facs Canto II & BD Leucocount	21	7.69	8.38	8.93	14.80

Staining Method Analysis	No. In Group	Lower Quartile	Median	Upper Quartile	CV%
BD Leucocount	74	7.05	7.90	8.70	20.87
Propidium Iodide	6	6.97	7.71	8.00	13.39
Coulter DNA-Prep	5	8.01	8.10	9.15	14.02
Leukosure	9	4.49	6.30	8.00	55.71

Issue Date: 17 February 2014

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Low Level Leucocytes Programme

Sample - RBC556

All Participant Report

Distribution - 151605

Participant ID -

Date Issued - 28 September 2015

Closing Date - 16 October 2015

Machine Used - Facs Canto II

Trial Comments

This trial was issued to 100 participants

Sample Comments

The sample was manufactured by UK NEQAS using a sample of filtered blood from an anonymous donor which was stabilised and spiked with stabilised buffy coat

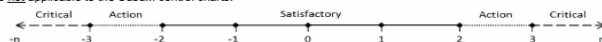
Results and Performance

Cell Population	Your Results (cells/ μ L)	Robust Mean (cells/ μ L)	Robust SD (cells/ μ L)
Machine 1	1.99	1.84	0.32

Cell Population	z Score*	Performance Status for this Sample	Performance Status Classification Over 12 Sample Period		
			Satisfactory	Action	Critical
Machine 1	0.47	Satisfactory	11	1	0

*z Score Limits Definitions

Please note the scale below is applicable to the tables above and to the z score histograms and Shewhart control charts that follow. It is not applicable to the Cusum control charts.



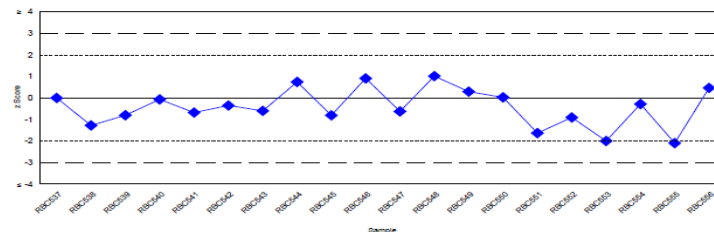
Low Level Leucocytes Programme

Sample - RBC556

Shewhart Control Charts

(Please note each data point represents a single sample)

Absolute Values (cells/uL) - Machine 1



Cusum Control Charts

(Please note each data point represents the sum of the z scores of the current sample and the two previous samples)

Absolute Values (cells/ μ L) - Machine 1

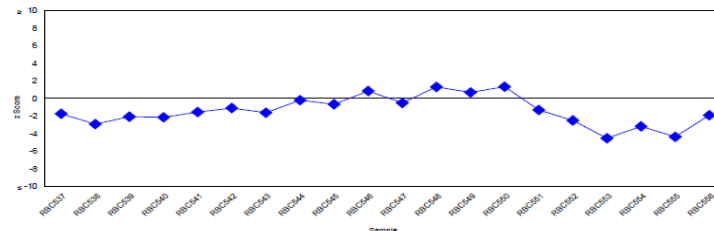


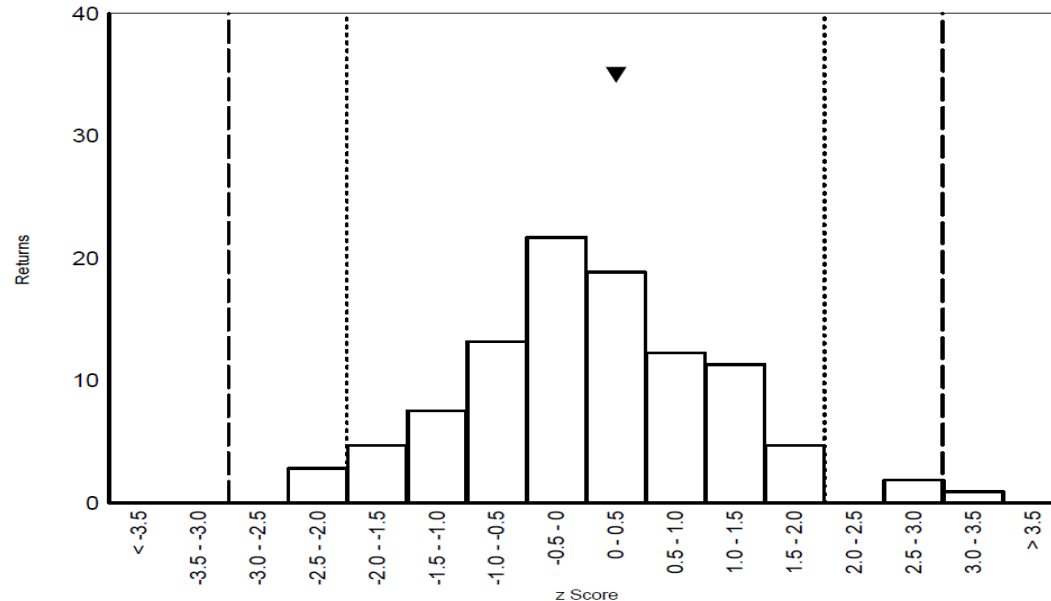
Chart Types

- Performance histogram
- Shewhart control charts
- Cusum control charts

Performance histogram compares participant results to overall consensus results on one trial for one analyte

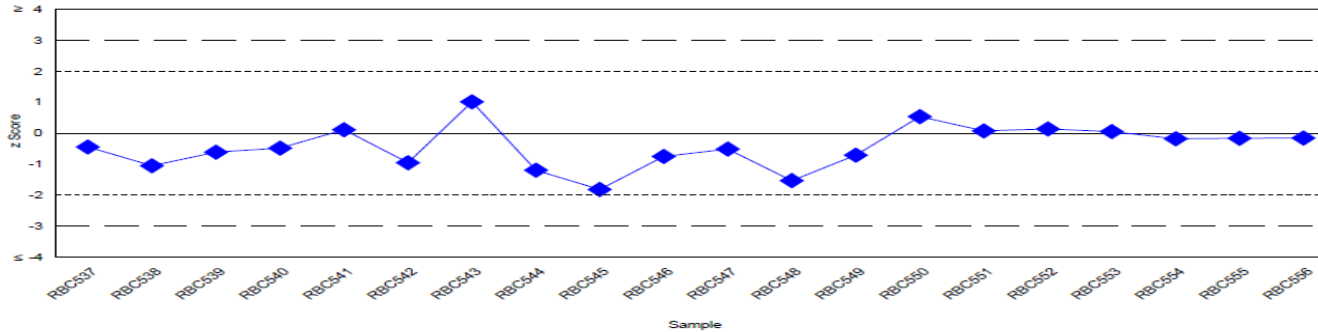
Histograms of Participant z Scores

Absolute Values (cells/ μ L) - Machine 1
Please note ▼ denotes your result

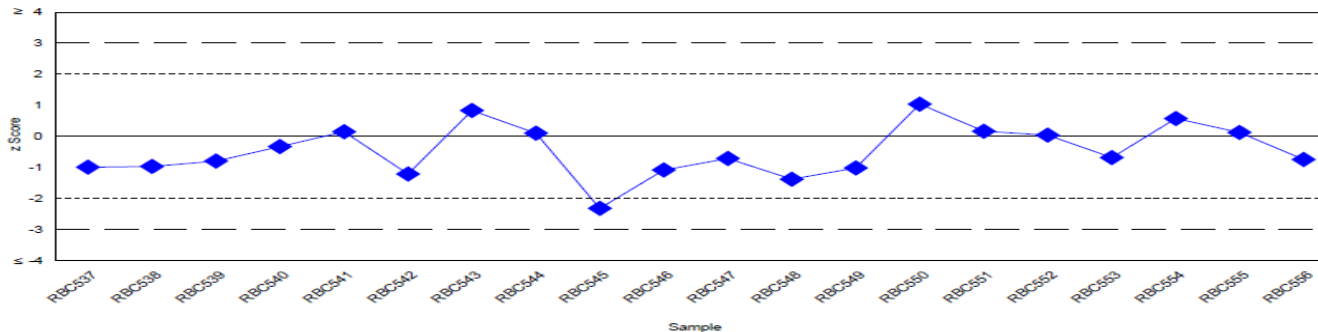


Shewhart control charts identify problems causing erratic values of z scores

Absolute Values (cells/ μ L) - Machine 1

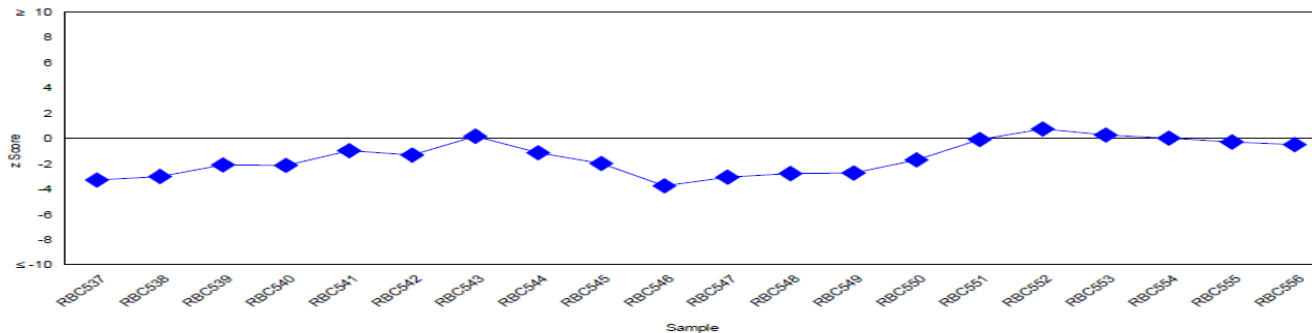


Absolute Values (cells/ μ L) - Machine 2

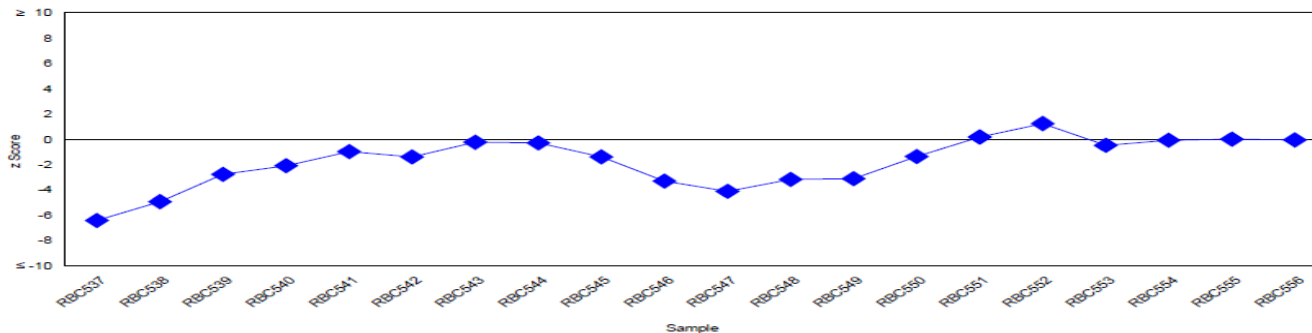


Cusum control charts identify problems causing a bias that persists over several rounds

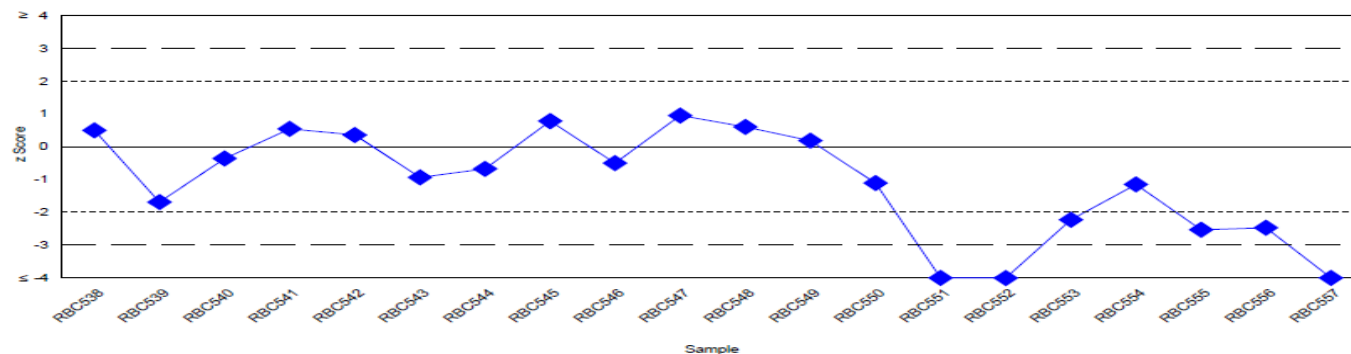
Absolute Values (cells/ μ L) - Machine 1



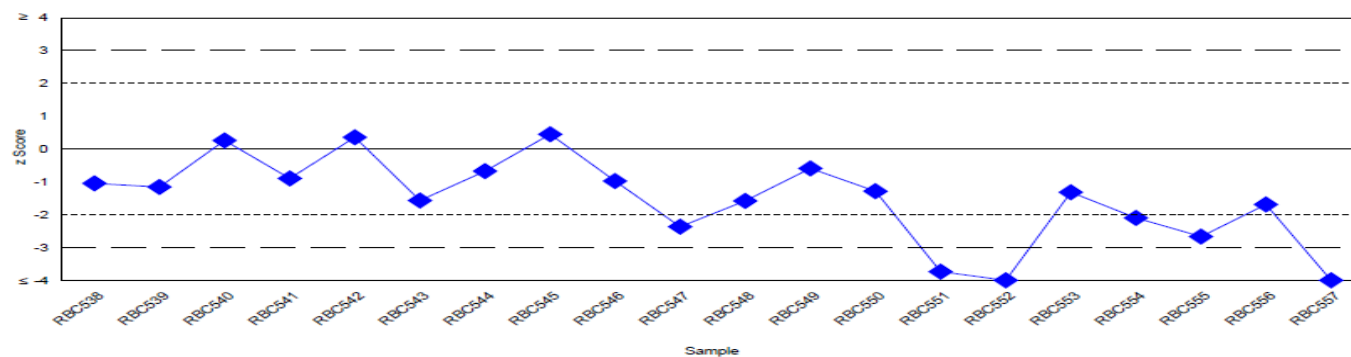
Absolute Values (cells/ μ L) - Machine 2



Absolute Values (cells/ μ L) - FC-500 MCL



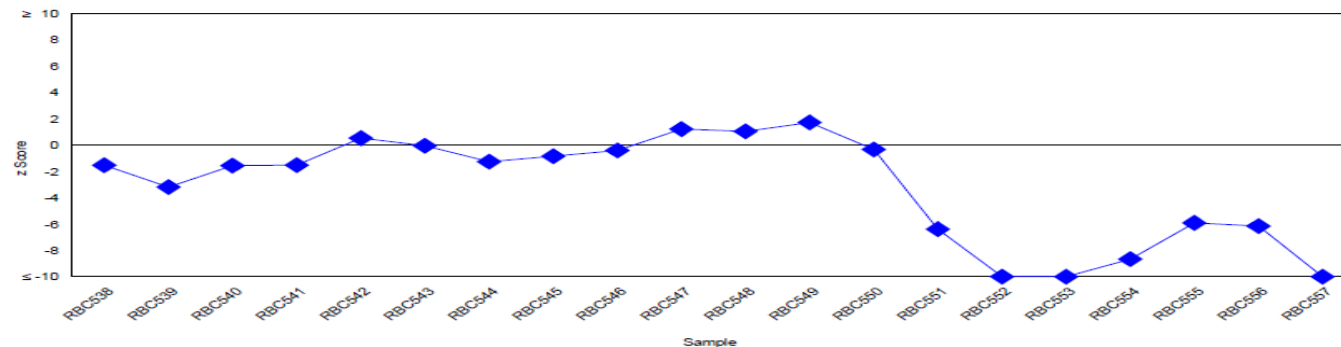
Absolute Values (cells/ μ L) - FC-500 MPL



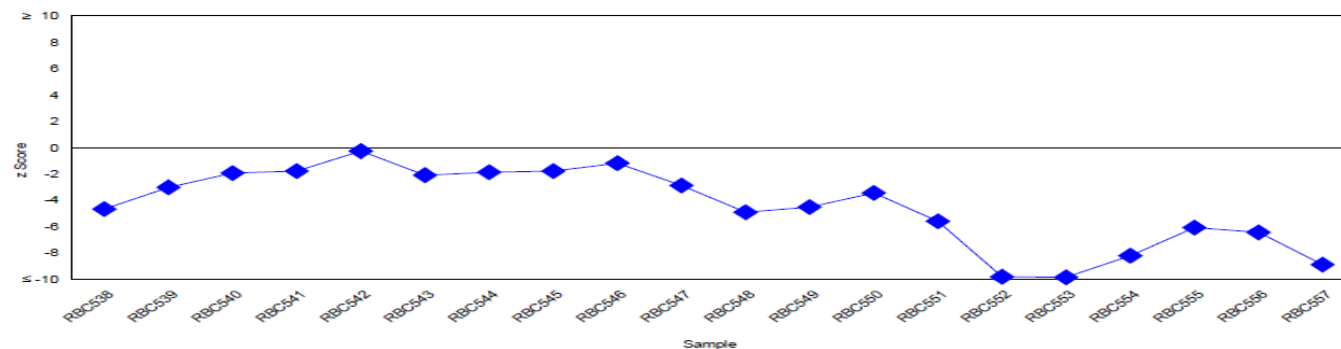
Cusum Control Charts

(Please note each data point represents the sum of the z scores of the current sample and the two previous samples)

Absolute Values (cells/ μ L) - FC-500 MCL



Absolute Values (cells/ μ L) - FC-500 MPL



Tables

- Tables provided are broken down by:
 - Cytometer model

Flow Cytometer Specific Statistics

(Please note only groups of >20 returns are displayed)

Method	Absolute Count Values (cells/ μ L)		
	Returns	Robust Mean	Robust SD
Facs Canto II	25	1.89	0.22
Facscalibur	39	1.84	0.37

- Staining Method

Staining Method Specific Statistics

(Please note only groups of >20 returns are displayed)

Method	Absolute Count Values (cells/ μ L)		
	Returns	Robust Mean	Robust SD
BD Leucocount	78	1.86	0.29

- All data provided is in the form of robust statistics
- Laboratories can calculate own methodological biases based on z score

Flow cytometers used in the low level leucocyte enumeration programme

Becton Dickinson Cytometers	Number of users
FACSCalibur	56
FACSCanto II	32
FACSCan	5
FACSCanto	3


Beckman Coulter Cytometers	Number of users
Navios	17
FC-500	16
XL	4
Gallios	1

Staining methods used in the low level leucocyte enumeration programme

Staining Method	Number Of Users
BD Leucocount	65
Propidium Iodide	8
Coulter DNA-Prep	5
Leukosure	11

Further Improvements

- UK NEQAS for Leucocyte Immunophenotyping is a UKAS Accredited Proficiency Testing Provider. No. 7804
- Low Level Leucocyte Enumeration programme was in the scope of the inspection

Schedule of Accreditation Issued by United Kingdom Accreditation Service 21 - 47 High Street, Feltham, Middlesex, TW13 4UN, UK		
 7804 Accredited to ISO/IEC 17043:2010	Sheffield Teaching Hospitals NHS Foundation Trust, operating UK NEQAS for Leucocyte Immunophenotyping Issue No: 002 Issue date: 08 September 2015	
	4th Floor Pegasus House 463a Glossop Road Sheffield S10 2SD United Kingdom	Contact: Joanne Antcliff Tel: 01142 673 600 Fax: 01142 673 601 E-Mail: joanne.antcliff@ukneqas.co.uk Website: www.ukneqas.co.uk
Proficiency Tests provided from the above address only		
DETAIL OF ACCREDITATION		
Materials/Products	Scheme Name/Type of Test/Properties Measured	Scheme Protocols/Procedures/ Techniques Used
Stabilised whole human blood	Immune monitoring (Absolute and percentage lymphocyte subsets)	See www.ukneqas.co.uk for scheme details
Stabilised whole human blood	CD34+ Stem Cell Enumeration (Absolute CD34+ stem cell numbers subsets)	
Stabilised whole human blood	Leukaemia Immunophenotyping and diagnostic interpretation (Surface antigen expression and case diagnosis)	
Stabilised whole human blood	Low level leucocyte enumeration (Absolute leucocyte count)	
Stabilised whole human blood	Paroxysmal Nocturnal Haemoglobinuria (Size of PNH population, Clone presence /absence)	
Lyophilised cells	JAK2 p.Val617Phe (V617F) Mutation status (Presence of JAK2 V617F mutation)	
Blood or Lyophilised cells	IgH/TCR Clonality Status (Identification of IgH/TCR clones)	
Blood or Lyophilised cells	Post-SCT Chimerism Monitoring	
Lyophilised cells	BCR-ABL 1 Major Quantification (Level of BCR-ABL mutation)	

Assessment Manager: JRI

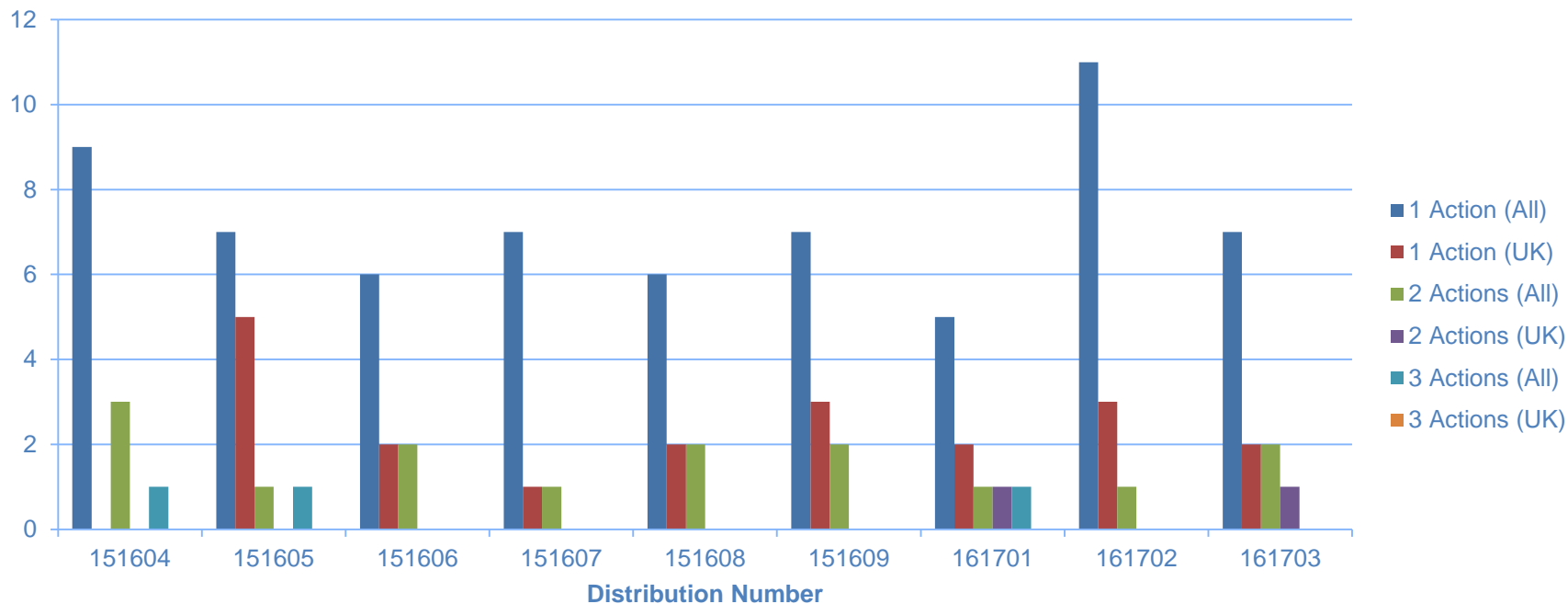
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Further Improvements

- Colour coded sample labels eg RBC 550, RBC 551, PLT 550, PLT 551 introduced
- Redesign of web page-reduction of data entry fields
- Addition of a limited free text comments field
- Use of weighted z-scores
- Review platelet sample formulation
- Provision of annual results summary

151609	RBC568	07-Mar-16	E97500520	-0.18471338	11	1	0	Satisfactory
151609	RBC569	07-Mar-16	E97500502	0.103448276	11	1	0	Satisfactory
151609	RBC569	07-Mar-16	E97500520	1.172413793	12	0	0	Satisfactory
151609	RBC570	07-Mar-16	E97500502	2.777777778	11	1	0	Action
151609	RBC570	07-Mar-16	E97500520	2.211111111	11	1	0	Action
151609	PLT568	07-Mar-16	Flow 1	16.08955224	10	1	1	Critical
151609	PLT568	07-Mar-16	Flow 2	18.11940299	11	0	1	Critical
151609	PLT569	07-Mar-16	Flow 1	-4.92517007	9	1	2	Critical
151609	PLT569	07-Mar-16	Flow 2	-5.00680272	10	0	2	Critical
151609	PLT570	07-Mar-16	Flow 1	-2.87333333	8	2	2	Action
151609	PLT570	07-Mar-16	Flow 2	-2.04	9	1	2	Action
151609	RBC568	07-Mar-16	Flow 1	-6.15286624	10	1	1	Critical
151609	RBC568	07-Mar-16	Flow 2	-6.40764331	10	1	1	Critical
151609	RBC569	07-Mar-16	Flow 1	12.31034483	9	1	2	Critical
151609	RBC569	07-Mar-16	Flow 2	16.22413793	9	1	2	Critical
151609	RBC570	07-Mar-16	Flow 1	5.888888889	9	0	3	Critical
151609	RBC570	07-Mar-16	Flow 2	7.533333333	8	1	3	Critical
151609	PLT568	07-Mar-16	E97300242	-0.59701493	12	0	0	Satisfactory
151609	PLT568	07-Mar-16	E97300283	-2.53731343	9	3	0	Action
151609	PLT569	07-Mar-16	E97300242	-0.85034014	12	0	0	Satisfactory
151609	PLT569	07-Mar-16	E97300283	-1.2585034	9	3	0	Satisfactory
151609	PLT570	07-Mar-16	E97300242	-1.53333333	12	0	0	Satisfactory
151609	PLT570	07-Mar-16	E97300283	-1.26666667	9	3	0	Satisfactory
151609	RBC568	07-Mar-16	E97300242	-6.50318471	10	0	2	Critical
151609	RBC568	07-Mar-16	E97300283	-6.12101911	10	0	2	Critical
151609	RBC569	07-Mar-16	E97300242	-3.63793103	9	0	3	Critical
151609	RBC569	07-Mar-16	E97300283	-3.98275862	9	0	3	Critical
151609	RBC570	07-Mar-16	E97300242	-7	8	0	4	Critical
151609	RBC570	07-Mar-16	E97300283	-6.11111111	8	0	4	Critical

Number of 'action' notifications per distribution (RBC's)



Number of 'action' notifications per distribution (PLT's)

