

## FLT3 Mutation Status Programme

Distribution - 151601 

Participant ID - 

Date Issued - 22 July 2015 

Closing Date - 21 August 2015 

### Trial Comments

The trial was issued to 100 participants for FLT3 analysis. 97 (97.0%) participants returned results; 3 did not, 1 of whom prenotified us of their non return.

### Sample Comments

Two vials of lyophilised cell line samples were manufactured and issued by UKNEQAS LI (sample refs FLT3 120 and FLT3 121), both positive for different FLT3 ITD mutations.

### Results and Performance

#### Your Results

| FLT3 Mutation Status                                                                              | Your Results                                                                                        | Consensus Result                                                                                      |
|---------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| Sample FLT3 120  | Mutation Detected  | Mutation Detected  |
| Sample FLT3 121  | Mutation Detected  | Mutation Detected  |

#### All Participant Results

|                 | Mutation Detected (Returns) | No Mutation Detected (Returns) |
|-----------------|-----------------------------|--------------------------------|
| Sample FLT3 120 | 95                          | 2                              |
| Sample FLT3 121 | 89                          | 8                              |

#### Your Performance

| Performance | Performance Status for this trial                                                                | Performance Status Classification Over 3 Trial Period                                  |                                                                                         |
|-------------|--------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
|             |                                                                                                  | Satisfactory                                                                           | Critical                                                                                |
|             | Satisfactory  | 3  | 0  |

N/A = Not Applicable

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### Template

|      | Returns |
|------|---------|
| DNA  | 79      |
| cDNA | 18      |

### PCR Type

|               | Returns |
|---------------|---------|
| Single PCR    | 69      |
| Multiplex PCR | 25      |
| Real-Time PCR | 2       |
| Sequencing    | 1       |

### Protocol Type

|                                            | Returns |
|--------------------------------------------|---------|
| In-house Assay                             | 81      |
| Leukostrat FLT3 Mutation Assay             | 5       |
| Invivoscribe FLT3 (Labelled or Unlabelled) | 4       |
| Molecular Diagnostic.be                    | 4       |
| Ampli Set FLT3 ITD                         | 1       |
| Ion AmpliSeq Cancer Hotspot Panel v2       | 1       |
| Truseq Myeloid Sequencing Panel            | 1       |

### Analysis Type

|                                       | Returns |
|---------------------------------------|---------|
| Capillary Electrophoresis             | 68      |
| Agarose Gel Electrophoresis           | 21      |
| Melt Curve Analysis                   | 2       |
| Next Generation Sequencing            | 2       |
| Sanger Sequencing                     | 2       |
| Acrylamide Gel Electrophoresis (PAGE) | 1       |
| Microfluidic Electrophoresis          | 1       |

## FLT3 Mutation Status Programme

### Journal Reference for Assay

|                                                          | Returns |
|----------------------------------------------------------|---------|
| Murphy KM et al (2003) J Mol Diagn 5(2):96-102           | 19      |
| Thiede C et al (2002) Blood 99(12):4326-4335             | 14      |
| Nakao M et al (1996) Leukemia 10(2):1911-1918            | 13      |
| Huang Q et al Br J Haematol (2008) 142 (3):489-492       | 8       |
| Kottaridis PD et al (2001) Blood 98(6):1752-1759         | 8       |
| Kiyoi H et al (1997) Leukemia 11(9):1447-1452            | 7       |
| Gale RE et al (2008) Blood 111(5):2776-2784              | 6       |
| Yamamoto Y et al (2001) Blood 97(8):2434-2439            | 5       |
| In-House Assay (no published reference available)        | 4       |
| Noguera NI et al (2005) Leukemia 19(18):1479-1482        | 4       |
| Schnittger S et al (2002) Blood 100(1):59-66             | 4       |
| Kiyoi H et al (1999) Blood 93(9):3074-3080               | 3       |
| Schnittger S et al (2011) Haematologica 96(12):1799-1807 | 3       |
| Bianchini M et al (2003) Clin Chem 49(10):1642-1650      | 2       |
| Kottaridis PD et al (2002) Blood 100(7):2393-2399        | 2       |
| MolecularDiagnostics.be assay                            | 2       |
| Tan AY et al (2008) J Haematol Oncol 1:10                | 2       |

### **FLT3 Mutation Status Programme**

#### **FLT3 ITD sample 120:**

- 95 out of 97 participants (97.9%) that returned results detected a *FLT3* internal tandem duplication (ITD) in sample FLT3 120.
- 2 (2.1%) participants failed to detect the ITD mutation; 1 used single PCR and the Leukostrat FLT3 Mutation assay and the other participant used a multiplex PCR and an in-house assay. Both participants used agarose gel detection.
- 76 participants provided the size of the ITD(s) detected in sample 120. 73 participants found a single ITD and 3 participants found 2 ITDs.
- Of the participants who identified a single ITD, 30 participants (41.1%) reported that it was 30bp in size. This result represented the modal and median value, and was in line with sample formulation expectations. ITD sizes reported ranged from 10-362bp.

#### **FLT3 ITD sample 121:**

- 89 out of 97 participants (90.7%) that returned results detected a *FLT3* internal tandem duplication (ITD) in sample FLT3 121.
- 8 participants (8.2%) participants failed to detect the ITD mutation. 3 of the laboratories utilised a single/multiplex PCR in combination with agarose gel electrophoresis. 2 laboratories employed a Next Generation Sequencing (NGS) approach. 2 participants used single PCR with capillary electrophoresis analysis. A further participant used a screening melt curve analysis.
- 71 participants provided the size of the ITD(s) detected in sample 121. 68 participants found a single ITD, 1 participant found 2 ITDs and 2 participants found 4 ITDs.
- Of the participants who identified a single ITD, 33 laboratories (46.4%) reported that it was 126bp in size. Again, this result represented the modal and median value, and was in line with sample formulation expectations. Results returned for the ITD size ranged from 20bp to 510bp.
- As was observed in trial FLT3 141503, NGS based approaches have difficulty in detecting large ITDs. As *FLT3* ITDs in AML typically range from approximately 15-153bp<sup>1</sup> with ITDs >400bp reported<sup>2</sup> this is problematic.
- Standard NGS analysis software for detecting insertions make insertion calls based on data obtained during the initial read mapping and alignment process. Because of the difficulty associated with aligning short reads, only small insertion events can be identified by this approach; short reads containing small insertions retain sufficient homology in the regions flanking the insertion to permit accurate alignment; large insertions do not. Pair-end based analysis approaches have been developed that can reliably detect larger insertions, such as the *FLT3* ITD<sup>3</sup>.

### **FLT3 Mutation Status Programme**

#### **Reference**

1. Stirewalt, D. L. Size of FLT3 internal tandem duplication has prognostic significance in patients with acute myeloid leukemia. *Blood* **107**, 3724–3726 (2006).
2. Meshinchi, S. & Appelbaum, F. R. Structural and functional alterations of FLT3 in acute myeloid leukemia. *Clin. Cancer Res.* **15**, 4263–9 (2009).
3. Spencer, D. H. *et al.* Detection of FLT3 Internal Tandem Duplication in Targeted, Short-Read-Length, Next-Generation Sequencing Data. *J. Mol. Diagnostics* **15**, 81–93 (2013).

Website only: Uncontrolled

## **FLT3 Mutation Status Programme**

### **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, 4<sup>th</sup> Floor Suite  
463A Glossop Road  
Sheffield, S10 2QD  
United Kingdom  
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601  
e-mail: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:

Prof David Barnett, Director or Mr Liam Whitby, Operations 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](http://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](http://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.