The t(1;19)(q23;p13) is the most common TCF3 rearrangement, being present in around 6% of childhood B-ALL. According to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukaemia, B lymphoblastic leukaemia/lymphoma with t(1;19) (q23;p13); TCF3-PBX1 is recognised as a distinct disease entity. The functional fusion gene resides at chromosome 19. An unbalanced form of this translocation has been reported, with loss of der(1)

Detection of the E2A-PBX1 fusion by molecular methods, such as FISH, is important, as a subset of B-ALLs has a karyotypically identical t(1;19) that involves neither TCF3 nor PBX1. E2A-PBX1 positive leukaemia was historically associated with a poor outcome, though modern intensive therapies have overcome this.

The t(17;19)(q22;p13) is a rare translocation that is present in around 1% of precursor B-ALL cases. TCF3-HLF positive leukaemia is associated with adverse prognosis.

Materials Provided

Probe: 50µl per vial (5 tests) or 100µl per vial (10 tests)

The probes are provided premixed in hybridisation solution (formamide; dextran sulphate; saline-sodium citrate (SSC)) and are ready to use.

Counterstain 150µl per vial (15 tests)

The counterstain is DAPI antifade (ES: 0.125µg/ml DAPI (4,6-diamidino-2-phenylindole)).

Warnings and Precautions

1. For in vitro diagnostic use. For professional use only.

2. Wear gloves when handling DNA probes and DAPI counterstain.

3. Probe mixtures contain formamide, which is a teratogen; do not breathe fumes or allow skin contact. Handle with care; wear gloves and a lab coat.

4. DAPI is a potential carcinogen. Handle with care; wear gloves and a lab coat.

5. Dispose of all hazardous materials according to your institution’s guidelines for hazardous waste disposal.

6. Operators must be capable of distinguishing the colours red, blue, and green.

7. Failure to adhere to the outlined protocol and reagents may affect the performance and lead to false positive/negative results.

8. The probe should not be diluted or mixed with other probes.

9. Failure to use 10µl of probe during the pre-denaturation stage of the protocol may affect the performance and lead to false positive/negative results.

Storage and Handling

The Aquarius® kit should be stored between -25°C to -15°C in a freezer until the expiry date indicated on the kit label. The probe and counterstain vials must be stored in the dark.

The probe remains stable throughout the freeze-thaw cycles experienced during normal use (where one cycle constitutes the probe’s removal from and replacement into the freezer) and is photostable for up to 48 hours after being exposed to continuous lighting conditions. All efforts must be made to limit exposure to light and temperature changes.

E2A (TCF3) Breakapart Probe

The E2A product consists of a 189kb probe, labelled in red, located centrometric to the E2A (TCF3) gene, including the RH98588 marker, and a green probe covering a 163kb region located telomeric to the E2A gene, including the D19S883 marker.

Probe Information

The TCF3 (transcription factor 3) gene is located at 19p13.3. Translocations involving TCF3 are some of the most common rearrangements in childhood B-cell acute lymphoblastic leukaemia (ALL).

Two of the main TCF3 partners are PBX1 (PBX homeobox 1) at 1q23.3 and HLF (HLF transcription factor, PAR2ZIP family member) at 17q22. These become fused to TCF3 as a result of the t(1;19)(q23;p13) and t(17;19)(q22;p13) translocations, forming the TCF3-PBX1 and TCF3-HLF fusion genes, respectively. A rare cryptic inversion, inv(19)(p13q13), has been reported to fuse TCF3 to TFP (TCF3 fusion partner) resulting in the TCF3-TP fusion gene.

The t(1;19)(q23;p13) is the most common TCF3 rearrangement, being present in around 6% of childhood B-ALL. According to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukaemia, B lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13); TCF3-PBX1 is recognised as a distinct disease entity. The functional fusion gene resides at chromosome 19. An unbalanced form of this translocation has been reported, with loss of der(1). Detection of the E2A-PBX1 fusion by molecular methods, such as FISH, is important, as a subset of B-ALLs has a karyotypically identical t(1;19) that involves neither TCF3 nor PBX1. E2A-PBX1 positive leukaemia was historically associated with a poor outcome, though modern intensive therapies have overcome this.

The t(17;19)(q22;p13) is a rare translocation that is present in around 1% of precursor B-ALL cases. TCF3-HLF positive leukaemia is associated with adverse prognosis.

Probe Specification

E2A, 19p13.3, Red
E2A, 19p13.3, Green
Equipment and Materials Necessary but not Supplied
Calibrated equipment must be used:
1. Hotplate (with a solid plate and accurate temperature control up to 80°C)
2. Calibrated variable volume micropipettes and tips range 1µl - 200µl
3. Water bath with accurate temperature control at 37°C and 72°C
4. Microcentrifuge tubes (0.5ml)
5. Fluorescence microscope (Please see Fluorescence Microscope Recommendation section)
6. Phase contrast microscope
7. Clean plastic, ceramic or heat-resistant glass Coplin jars
8. Forceps
9. Calibrated pH meter (or pH indicator strips capable of measuring pH 6.5 – 8.0)
10. Humidified container
11. Fluorescence grade microscope lens immersion oil
12. Bench top centrifuge
13. Microscope slides
14. 24x24mm coverslips
15. Timer
16. 37°C incubator
17. Rubber solution glue
18. Forceps
19. Graduated cylinders
20. Magnetic stirrer
21. Calibrated thermometer

Optional Equipment not Supplied
1. Cytogenetic drying chamber
2. 20x saline-sodium citrate (SSC) Solution
3. 100% Ethanol
4. 3-Methylhydroxy (NaOH)
5. 1M Hydrochloric acid (HCl)
6. Purified water

Fluorescence Microscope Recommendation
Use a 100-watt mercury lamp or equivalent and oil immersion plan apochromat objectives 60x/63x or 100x for optimal visualisation. The fluorophores used in this probe set will excite and emit at the following wavelengths:

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Excitation max [nm]</th>
<th>Emission max [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>495</td>
<td>521</td>
</tr>
<tr>
<td>Red</td>
<td>596</td>
<td>615</td>
</tr>
</tbody>
</table>

Ensure appropriate excitation and emission filters that cover the wavelengths listed above are fitted to the microscope. Use a triple bandpass DAPI/green spectrum filter or a dual bandpass green spectrum/red spectrum filter for optimal simultaneous visualisation of the green and red fluorophores.

Check the fluorescence microscope before use to ensure it is operating correctly. Use immersion oil that is suitable for fluorescence microscopy and formulated for low autofluorescence. Avoid mixing DAPI antifade with microscope immersion oil as this will obscure signals. Follow manufacturers’ recommendations in regards to the life of the lamp and the age of the filters.

Sample Preparation
The kit is designed for use on haematologically-derived cell suspensions fixed in Carnoy’s solution (3:1 methanol/acetic acid) fixative, that are prepared according to the laboratory or institution guidelines. Prepare air dried samples on microscope slides according to standard cytogenetic procedures. The AGT Cytogenetics Laboratory Manual contains recommendations for specimen collection, culturing, harvesting and for slide making.

Solution Preparation

**Ethanol Solutions**
Dilute 100% ethanol with purified water using the following ratios and mix thoroughly:
- 70% Ethanol - 7 parts 100% ethanol to 3 parts purified water
- 85% Ethanol - 8.5 parts 100% ethanol to 1.5 parts purified water
Store the solutions for up to 6 months at room temperature in an airtight container.

**2xSSC Solution**
Dilute 1 part 20xSSC Solution with 9 parts purified water and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

**0.4xSSC Solution**
Dilute 1 part 20xSSC Solution with 49 parts purified water and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

**2xSSC, 0.05% Tween-20 Solution**
Dilute 1 part 20xSSC Solution with 9 parts purified water. Add 5µl of Tween-20 per 10ml and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

**FISH Protocol**
(Note: Ensure that exposure of the probe and counterstain to laboratory lights is limited at all times.)

**Slide Preparation**
1. Spot the cell sample onto a glass microscope slide. Allow to dry (Optional, if using a cytogenetic drying chamber: slides should be spotted using a cytogenetic drying chamber. The chamber should be operated at approximately 25°C and 50% humidity for optimal cell sample spotting. If a cytogenetic drying chamber is not available, use a fume hood as an alternative).
2. Immers the slide in 2xSSC for 2 minutes at room temperature (RT) without agitation.
3. Dehydrate in an ethanol series (70%, 85% and 100%), each for 2 minutes at RT.
4. Allow to dry.

**Pre-Denaturation**
5. Remove the probe from the freezer and allow it to warm to RT. Briefly centrifuge tubes before use.
6. Ensure that the probe solution is uniformly mixed with a pipette.
7. Remove 10µl of probe per test, and transfer it to a microcentrifuge tube. Quickly return the remaining probe to the freezer.
8. Place the probe and the sample slide to prewarm at a 37°C (+/- 1°C) hotplate for 5 minutes.
9. Spot 10µl of probe mixture onto the cell sample and carefully apply a coverslip. Seal with rubber solution glue and allow the glue to dry completely.

**Denaturation**
10. Denature the sample and probe simultaneously by heating the slide on a hotplate at 75°C (+/- 1°C) for 2 minutes.

**Hybridisation**
11. Place the slide in a humid, lightproof container at 37°C (+/- 1°C) overnight.

**Post-Hybridisation Washes**
12. Remove the DAPI from the freezer and allow it to warm to RT.
13. Remove the coverslip and all traces of glue carefully.
14. Immers the slide in 0.4xSSC (pH 7.0) at 72°C (+/- 1°C) for 2 minutes without agitation.
15. Drain the slide and immerse it in 2xSSC, 0.05% Tween-20 at RT (pH 7.0) for 30 seconds without agitation.
16. Drain the slide and apply 10µl of DAPI antifade onto each sample.
17. Cover with a coverslip, remove any bubbles and allow the colour to develop in the dark for 10 minutes.
18. View with a fluorescence microscope (see Fluorescence Microscope Recommendation).

**Stability of Finished Slides**
Finished slides remain analysable for up to 1 month if stored in the dark after RT.

**Procedural Recommendations**
1. Baking or ageing of slides may reduce signal fluorescence
2. Hybridisation conditions may be adversely affected by the use of reagents other than those provided or recommended by Cytocell Ltd
3. Use a calibrated thermometer for measuring temperatures of solutions, waterbaths and incubators as these temperatures are critical for optimum product performance.
4. The wash concentrations, pH and temperatures are important as low stringency can result in non-specific binding of the probe and too high stringency can result in a lack of signal
5. Incomplete denaturation can result in lack of signal and over denaturation can also result in non-specific binding
6. Over hybridisation can result in additional or unexpected signals
7. Users should optimise the protocol for their own samples prior to using the test for diagnostic purposes
8. Suboptimal conditions may result in non-specific binding that may be misinterpreted as a probe signal

**Interpretation of Results**

**Assessing Slide Quality**
The slide should not be analysed if:
- Signals are too weak to analyse in single filters - in order to proceed with analysis, signals should appear bright, distinct and easily evaluable
- There are high numbers of clumped/overlapping cells obstructing the analysis
- >50% of the cells are not hybridised
- There is excess of fluorescent particiles between cells and/or a fluorescent haze that interferes with the signals - in optimal slides the background should appear dark or black and clean
- Cell nucleus borders cannot be distinguished and are not intact

**Analysis Guidelines**
- Two analysts should analyse and interpret each sample. Any discrepancies should be resolved by assessment by a third analyst
- Each analyst should be suitably qualified according to recognised national standards
- Each analyst should score independently 100 nuclei for each sample. The first analyst should start the analysis from the left side of the slide and the second analyst from the right side
- Each analyst should document their results in separate sheets

DSO77/CE-en v011.00/2020-12-01 (H020 v2)
• Analyse only intact nuclei, not overlapped or crowded nuclei or nuclei covered by cytoplasmic debris or high degree of autofluorescence
• Avoid areas where there is excess of cytoplasmic debris or non-specific hybridisation
• Signal intensity may vary, even with a single nucleus. In such cases, use single filters and/or adjust the focal plane
• In suboptimal conditions signals may appear diffuse. If two signals of the same colour touch each other, or the distance between them is no greater than two signal widths, or when there is a faint strand connecting the two signals, count as one signal
• When analysing dual-colour breakapart probes, if there is a gap between the red and green signal no greater than two signal widths apart, count as not rearranged/fused signal
• If in doubt about whether a cell is analysable or not, then do not analyse it
• If applicable, the event should also be reported to your national competent authority. A list of vigilance contact points can be found at http://ec.europa.eu/growth/sectors/medical-devices/contacts/

Analysis Guidelines

| Do not count – nuclei are too close together to determine boundaries |
| Do not count overlapping nuclei – all areas of both nuclei are not visible |
| Count as two fusion signals - the gap between the red and green signal is less than two signal widths |
| Count as two fusion signals - one fusion signal is diffuse |

Expected Results

Expected normal signal pattern

In a normal cell, two red/green fusion signals are expected (2F).

Expected abnormal signal pattern

In a cell with a balanced E2A (TCF3) rearrangement, the expected signal pattern will be one red, one green and one fusion (1R, 1G, 1F).

Other signal patterns are possible in aneuploid/unbalanced specimens.

Known Cross-Reactivity

No known cross-reactivity.

Adverse Event Reporting

If you believe this device has malfunctioned or suffered a deterioration in its performance characteristics which may have contributed to an adverse event (e.g. delayed or misdiagnosis, delayed or inappropriate treatment), this must be reported immediately to the manufacturer (email: vigilance@ogt.com).

If applicable, the event should also be reported to your national competent authority. A list of vigilance contact points can be found at http://ec.europa.eu/growth/sectors/medical-devices/contacts/.

Specific Performance Characteristics

Analytical Specificity

Analytical specificity is the percentage of signals that hybridise to the correct locus and no other location. The analytical specificity was established by analysing a total of 200 target loci. The analytical specificity was calculated as the number of FISH signals that hybridised to the correct locus divided by the total number of FISH signals hybridised.

Table 1. Analytical Specificity for the E2A Breakapart Probe

<table>
<thead>
<tr>
<th>Probe</th>
<th>Target Locus</th>
<th>No. of Signals Hybridised to the Correct Locus</th>
<th>Total No. of Signals Hybridised</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red E2A</td>
<td>19p13</td>
<td>200</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Green E2A</td>
<td>19p13</td>
<td>200</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

Analytical Sensitivity

Analytical sensitivity is the percentage of scoreable interphase cells with the expected normal signal pattern. The analytical sensitivity was established by analysing interphase cells across different normal samples. The sensitivity was calculated as the percentage of scoreable cells with the expected signal pattern (with a 95% confidence interval).

Table 2. Analytical Sensitivity for the E2A Breakapart Probe

<table>
<thead>
<tr>
<th>No. of Cells with Expected Signal Patterns</th>
<th>No. of Cells with Scoreable Signals</th>
<th>Sensitivity (%)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>386</td>
<td>400</td>
<td>96.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Characterisation of Normal Cut-off Values

The normal cut-off value, in association with FISH probes, is the maximum percentage of scoreable interphase cells with a specific abnormal signal pattern at which a sample is considered normal for that signal pattern.

The normal cut-off value was established using samples from normal and positive patients. For each sample, the signal patterns of 100 cells were recorded. The Youden index was calculated to find the threshold value for which Sensitivity + Specificity - 1 is maximised.

Table 3. Characterisation of Normal Cut-off Values for the E2A Breakapart Probe

<table>
<thead>
<tr>
<th>Abnormal signal pattern</th>
<th>Youden Index</th>
<th>Normal Cut-off (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1R, 1G, 1F</td>
<td>0.99</td>
<td>6</td>
</tr>
</tbody>
</table>

Precision and Reproducibility

Precision is a measure of the natural variation of a test when repeated several times under the same conditions. This was assessed by analysing repeats of the same lot number of probe tested on the same sample, in the same conditions on the same day.

Reproducibility is a measure of the variability of a test and has been established in terms of sample-to-sample, day-to-day and batch-to-batch variability. Day-to-day reproducibility was assessed by analysing the same samples on three different days. Batch-to-batch reproducibility was assessed by analysing the same samples using three different lot numbers of probe on one day. Sample-to-sample reproducibility was assessed by analysing three replicates of a sample on one day. For each sample, signal patterns of 100 interphase cells were recorded and the percentage of cells with the expected signal pattern was calculated.

The reproducibility and precision were calculated as the Standard Deviation (STDEV) between replicates for each variable and overall mean STDEV.

Table 4. Reproducibility and Precision for the E2A Breakapart Probe

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard Deviation (STDEV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>0.19</td>
</tr>
<tr>
<td>Sample-to-sample</td>
<td>0.19</td>
</tr>
<tr>
<td>Day-to-day</td>
<td>0.38</td>
</tr>
<tr>
<td>Batch-to-batch</td>
<td>0.00</td>
</tr>
<tr>
<td>Overall deviation</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Clinical Performance

The clinical performance was established on a representative sample of the intended population for the product. For each sample, the signal patterns of ≥100 interphase cells were recorded. A normal/abnormal determination was made by comparing the percentage of cells with the specific abnormal signal pattern to the normal cut-off value. The results were then compared to the known status of the sample.

The results of the clinical data were analysed in order to produce sensitivity, specificity and cut-off values using a one-dimensional approach.
### Table 5. Clinical Performance for the E2A Breakapart Probe

<table>
<thead>
<tr>
<th>Variable</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Sensitivity (true positive rate, TPR)</td>
<td>100%</td>
</tr>
<tr>
<td>Clinical Specificity (true negative rate, TNR)</td>
<td>99.8%</td>
</tr>
<tr>
<td>False Positive rate (FPR) = 1 − Specificity</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

**Additional Information**
For additional product information please contact the CytoCell Technical Support Department.

T: +44 (0)1223 294048
E: techsupport@cytocell.com
W: www.ogt.com

**References**

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**Guide to Symbols**

- **REF**: Catalogue number
- **IVD**: In vitro diagnostic medical device
- **LOT**: Batch code
- **I**: Consult instructions for use
- **M**: Manufacturer
- **U**: Use-by date
- **T**: Temperature limit
- **K**: Keep away from sunlight
- **C**: Contains sufficient for <n> tests
- **CONT**: Contents

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**Patents and Trademarks**

CytoCell is a registered trademark of Cytocell Ltd.

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