Vysis CLL FISH Probe Kit

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REF 04N02-021

30-608712/R1

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Key to Symbols Used	
REF	Reference Number
LOT	Lot Number
IVD	In Vitro Diagnostic Medical Device
	Use By
	Temperature Limit
	Caution, consult accompanying documents
	Biological risks
	Consult instructions for use
	Contains sufficient for <n> tests
Rx ONLY	For Prescription Use Only
In Vitro Test	In Vitro Test
PRODUCT OF USA	Product of USA
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
GTIN	Global Trade Item Number
	Danger
	Danger
	Danger
	Danger
EC REP	Authorized Representative in the European Community
	Manufacturer

NAME

Vysis CLL FISH Probe Kit

INTENDED USE

The Vysis CLL FISH Probe Kit is a test to detect deletion of the LSI TP53, LSI ATM, and LSI D13S319 probe targets and gain of the D12Z3 sequence probe target via fluorescence in situ hybridization (FISH) in peripheral blood specimens from patients with B-cell chronic lymphocytic leukemia (CLL).

INDICATIONS FOR USE

In untreated patients, the assay may be used to dichotomize CLL (the 13q-, +12, or normal genotype group versus the 11q- or 17p- group) and may be used as an aid in determining disease prognosis in combination with additional biomarkers, morphology, and other clinical information.
The test is indicated for detecting deletion of the LSI TP53 probe target (17p-) as an aid in identifying those patients with CLL for whom treatment with VENCLEXTA® (venetoclax) is indicated.
Vysis CLL FISH Probe Kit is not intended for monitoring of residual disease.
The test is for prescription use only.

SUMMARY AND EXPLANATION OF THE TEST

Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in the developed world. Approximately 1 in 202 men and women will be diagnosed with CLL during their lifetime.[1] The median age at diagnosis is approximately 70 years of age. Incidence rates are higher for males (6.44 per 100,000 population) than females (3.51 per 100,000 population).[1] Leukemia incidence is highest among non-Hispanic whites (13.6 per 100,000 population); incidence is lowest among Asian and Pacific Islander populations (7.4 per 100,000 population) and American Indian and Alaska Native populations (7.3 per 100,000 population).[2]

Currently, most patients diagnosed with CLL have early stage-disease (Rai stage 0 or 1). Patients with early-stage CLL are a heterogeneous group; approximately 30% to 50% are at high risk of accelerated disease progression, and the remainder may live for decades and possibly never require therapy. Recent insights into the biological characteristics of leukemic B cells have led to the discovery of new prognostic tools (immunoglobulin variable-region heavy chain gene mutation status, cytogenetic abnormalities assessed by fluorescence in situ hybridization [FISH], and Z-chain-associated protein kinase-70 protein expression) that can contribute to the identification of patients with early-stage disease who are at high risk for early disease progression.[3]

Routine karyotype analysis only detects chromosomal aberrations associated with CLL in 40% to 50% of the cases. Use of FISH and other technologies have detected genomic abnormalities in over 80% of cases of CLL. The common genomic aberrations seen are trisomy 12 and deletions of 13q, 17p, and 11q.[4-6]

Several published studies suggest that some of these chromosomal abnormalities may be correlated with various disease parameters.[7-10]
The Vysis CLL FISH Probe Kit uses FISH DNA probe technology to determine deletion status of probe targets for locus-specific identifier (LSI) TP53 (containing tumor protein p53 gene, located on chromosome 17p), LSI ATM (containing ataxia telangiectasia mutated gene, located on chromosome 11q), and LSI D13S319 (containing marker D13S319, located on chromosome 13q), as well as determining trisomy 12 with CEP12 (D12Z3 alpha satellite, located on chromosome 12).

The Vysis CLL FISH Probe Kit includes LSI 13q34 (containing lysosomal-associated membrane protein 1 gene, located on chromosome 13q) as a control probe.

The National Comprehensive Cancer Network (NCCN) Practice Guidelines™ for Non Hodgkins Lymphoma (v.2.2016) which are the consensus recommendations of leading US oncology experts, states FISH (including abnormalities tested for by this kit) is informative for both prognosis and therapy determination. The guidelines recommend use of FISH at the time of diagnosis as well as re-evaluation by FISH at the time of relapse to direct treatment options (including abnormalities tested for by this kit).[11]

The tumor suppressor protein, p53, has been shown to play a critical role in oncogenesis and response to chemotherapy in a variety of human cancers. In humans the TP53 gene is found on the short arm of chromosome 17 (17p13)[12] and is reported to be suppressed or mutated in a large number of human cancers.[13] Deletions of the 17p region resulting in abnormalities of the tumor suppressor protein p53 have been identified as one of the poorest prognostic factors for CLL as it is predictive of short time to disease progression, short response duration, lack of response to therapy and short overall survival (OS).[7,14]

The 17p deletion is more frequently observed in treated patients than in previously untreated patients, increasing in frequency during the course of the disease with up to 50% of patients with relapsed or refractory disease having the deletion.[14] Approximately 8 to 12% of patients with CLL in the first line treatment carry del 17p.[14] It is widely accepted that treatment outcomes in patients with del 17p are poor.[15,16] Clinical trials of venetoclax in patients with refractory and relapsed CLL have shown that patients with 17p deletion are likely to experience clinical benefit. Refer to the VENCLEXTA® (venetoclax) label for more information.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

DNA Probe Description

Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen Probes

The SpectrumOrange-labeled LSI TP53 probe, approximately 172 Kb in length (chr17:7494395-7666098; February 2009 Assembly University of California, Santa Cruz [UCSC] Human Genome Browser[17]), is located at 17p13.1 and contains the complete TP53 gene.

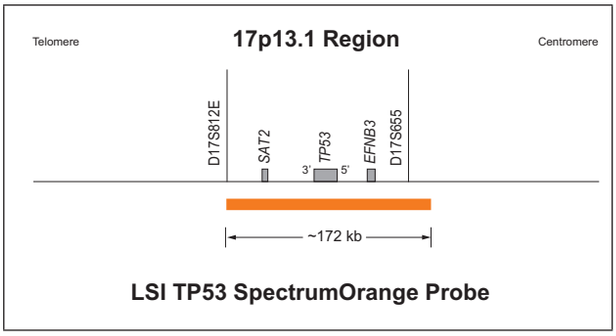
The SpectrumGreen-labeled LSI ATM probe, approximately 731 Kb in length (chr11:107801007-108531514; February 2009 Assembly UCSC Human Genome Browser), is located at 11q22.3 and contains the ATM gene.

Vysis LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen Probes

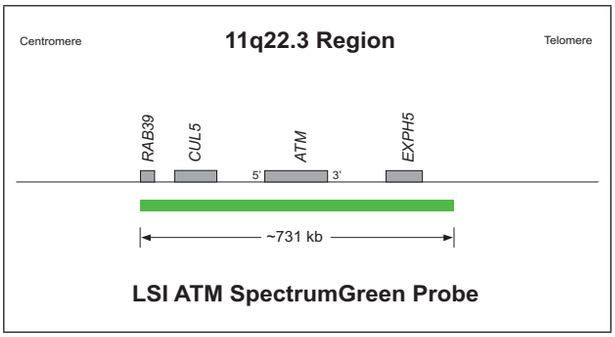
The SpectrumOrange-labeled LSI D13S319 probe, approximately 135 Kb in length (chr13:50602368-50737301; February 2009 Assembly UCSC Human Genome Browser), is located at 13q14.3.

The SpectrumAqua-labeled LSI 13q34 probe, approximately 612 Kb in length (chr13:113691322-114303435; February 2009 Assembly UCSC Human Genome Browser), is located at 13q34.

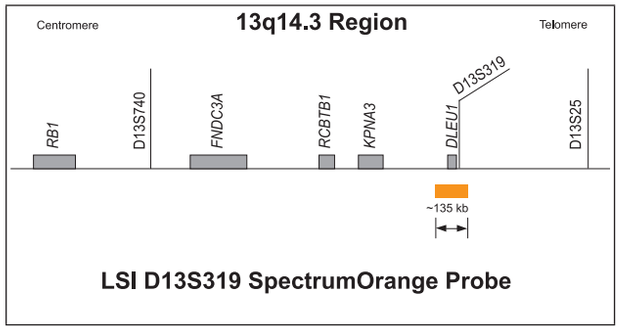
The SpectrumGreen-labeled CEP 12 probe is located at the centromere of chromosome 12.



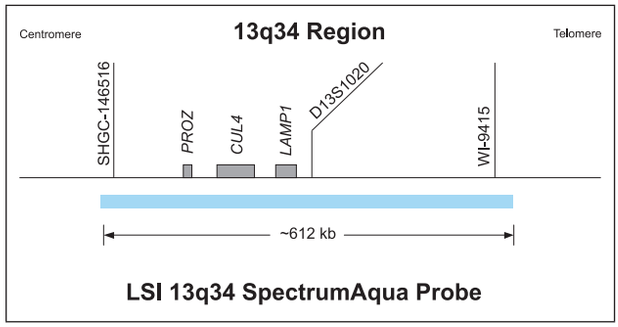
LSI TP53 SpectrumOrange Probe



LSI ATM SpectrumGreen Probe



LSI D13S319 SpectrumOrange Probe



LSI 13q34 SpectrumAqua Probe

General Reagents Description

DAPI II Counterstain

DAPI II Counterstain consists of DAPI (4′,6-diamidino-2 phenylindole•2HCl) (a DNA-specific fluorophore) and 1,4-phenylenediamine (an antifade compound used to reduce the tendency of the fluorophores to diminish in intensity) in a glycerol and phosphate buffered saline mixture.

NP-40

NP-40 is a non-ionic surfactant that is used in the aqueous post-hybridization wash solution.

20X Standard Sodium Citrate (SSC) Salt

20X SSC is a salt composed of sodium chloride and sodium citrate. It is used to make 20X SSC solution and subsequent dilutions for denaturing and wash solutions.

Technique Description

FISH is a technique that allows visualization of specific nucleic acid sequences within a cellular preparation. Specifically, FISH involves precise annealing of a single-stranded, fluorophore-labeled DNA probe to a complementary target sequence. Hybridization of the probe with the cellular DNA site is visible by direct detection using fluorescence microscopy.

Peripheral blood cells from CLL patients are attached to microscope slides using standard cytogenetic procedures. The resulting specimen DNA is denatured to single-stranded form and subsequently allowed to hybridize with the probes of the Vysis CLL FISH Probe Kit. Following hybridization, the unbound probe is removed by a series of washes, and the nuclei are counter-stained with DAPI, a DNA-specific stain that fluoresces blue. Hybridization of the Vysis LSI TP53 SpectrumOrange, LSI ATM SpectrumGreen, LSI D13S319 SpectrumOrange, LSI 13q34 SpectrumAqua, and CEP 12 SpectrumGreen probes is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters, allowing visualization of the orange, green, and aqua fluorescent signals.

In a cell with normal copy numbers of the Vysis LSI TP53 SpectrumOrange and Vysis LSI ATM SpectrumGreen probe targets, two orange and two green signals will be expected. In a cell with normal copy numbers of Vysis LSI D13S319 SpectrumOrange and CEP 12 SpectrumGreen probe targets, two orange signals and two green signals will be expected. Enumeration of the Vysis LSI TP53 SpectrumOrange, LSI ATM SpectrumGreen, LSI D13S319 SpectrumOrange, and CEP12 SpectrumGreen signals provides a mechanism for determining absolute copy number of the probe targets and the presence of the aberrations of interest. The LSI 13q34 SpectrumAqua probe functions as an assay process control and is described further in the section on Quality Controls later in this document.

REAGENTS

Material Provided

This kit contains five reagents sufficient to process 20 assays. An assay is defined as one 22 mm x 22 mm Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen probe hybridization area and one 22 mm x 22 mm Vysis LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen probe hybridization area.

Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen Probes

Part No.	30-241051
Quantity	1 vial, 200 µL/vial (300 and 200 ng/10 µL)
Storage	–20°C (± 5°C) and protected from light
Composition	SpectrumOrange and SpectrumGreen fluorophore-labeled DNA probes in hybridization buffer

Vysis LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen Probes

Part No.	30-241050
Quantity	1 vial, 200 µL/vial (200, 400 and 25 ng/10 µL)
Storage	–20°C (± 5°C) and protected from light
Composition	SpectrumOrange, SpectrumAqua, and SpectrumGreen fluorophore-labeled DNA probes in hybridization buffer

DAPI II Counterstain

Part No.	30-804811
Quantity	1 vial, 600 µL/vial (125 ng/mL)
Storage	–20°C (± 5°C) and protected from light
Composition	DAPI (4′,6-diamidino-2-phenylindole•2HCl) and 1,4-phenylenediamine in a glycerol and phosphate buffered saline mixture

NP-40

Part No.	30-804810
Quantity	2 vials, 2000 µL/vial
Storage	–25°C to 30°C
Composition	NP-40 (non-ionic detergent)

20X SSC Salt

Part No.	30-804812
Quantity	1 bottle, 66 g
Storage	–25°C to 30°C
Composition	Sodium chloride and sodium citrate

REAGENT STORAGE AND HANDLING INSTRUCTIONS

The Vysis CLL FISH Probe Kit must be stored at -20°C (± 5°C) and protected from light when not in use.

The NP-40 and 20X SSC Salt may be stored separately at room temperature.

Shipping Conditions

The Vysis CLL FISH Probe Kit is shipped on dry ice.

If you receive reagents that are in a condition contrary to label recommendation, or that are damaged, contact Abbott Molecular Technical Services.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device.

For in vitro diagnostic use.

CAUTION: United States Federal Law restricts this device to sale and distribution to or on the order of a physician or to a clinical laboratory; use is restricted to, by, or on the order of a physician.

Safety Precautions

CAUTION: This preparation contains human sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[18] OSHA Standards on Bloodborne Pathogens,[19] CLSI Document M29-A3,[20] and other appropriate biosafety practices.[21] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[18]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state and federal regulations.[21]

Hazard-determining components of labeling:

- Formamide
- Polyethylene glycol octylphenyl ether

The following warnings apply:

Danger

H302	Harmful if swallowed.
H315	Causes skin irritation.
H318	Causes serious eye damage.
H411	Toxic to aquatic life with long lasting effects.

P280	Wear protective gloves / protective clothing / eye protection.
P264	Wash hands thoroughly after handling.
P273	Avoid release to the environment.

P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER/doctor.
P301+P312	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell.
P302+P352	IF ON SKIN: Wash with plenty of water.
P332+P313	If skin irritation occurs: Get medical advice/ attention.
P330	Rinse mouth.
P362	Take off contaminated clothing.
P501	Dispose of contents / container in accordance with local regulations.

Danger

H360	May damage fertility or the unborn child.
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves / protective clothing / eye protection.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheet Statement: Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet.

NOTE: Safety Data Sheets (SDS) for all reagents provided in the kits are available upon request from the Abbott Molecular Technical Service Department.

Assay Precautions

- The Vysis CLL FISH Probe Kit is only for use with specimens that have been handled and stored as described in this package insert.
- Do not use the Vysis CLL FISH Probe Kit beyond its Use By date.
- Package insert instructions must be followed. Failure to adhere to package insert instructions may yield erroneous results.
- Fluorophores are readily photo bleached by exposure to light. To limit this degradation, store slides and probe kits in the dark, and handle all slides and probe kits containing fluorophores in reduced light. This includes all steps involved in handling the hybridized slide. Carry out all steps which do not require light for manipulation (incubation periods, washes, etc.) in the dark or reduced light.
- Probe mixtures are formulated at intended use concentrations and should not be diluted.
- Always verify the temperature of the wash solutions prior to each use by measuring the temperature of the solution in the Coplin jar with a calibrated thermometer.

Laboratory Precautions

- All biological specimens should be treated as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all human specimens should be treated with universal precautions.
- Hemolyzed specimens may prevent proper culture for standard cytogenetic analysis. Exposure of the specimens to acids, strong bases, or extreme heat should be avoided. Such conditions are known to damage DNA and may result in FISH assay failure.
- Failure to follow all procedures for slide denaturation, hybridization, and detection may cause unacceptable or erroneous results.
- The DAPI II Counterstain contains DAPI and 1,4-phenylenediamine.
- DAPI is a possible mutagen based on positive genotoxic effects. Avoid inhalation, ingestion, or contact with skin. Refer to SDS for specific warnings.
- 1,4-phenylenediamine is a known dermal sensitizer and a possible respiratory sensitizer. Avoid inhalation, ingestion, or contact with skin. Refer to SDS for specific warnings.
- The Vysis CLL FISH Probe Kit contains formamide, a teratogen. Avoid contact with skin and mucous membranes.
- Calibrated thermometers are required for measuring temperatures of solutions and water baths.
- All hazardous materials should be disposed of according to the institution's guidelines for hazardous disposal.

ASSAY PROCEDURE

Materials Provided

- Vysis CLL FISH Probe Kit (List No. 04N02-021)

Materials Required But Not Provided

- Immersion oil appropriate for fluorescence microscopy
- Ethanol (100%). Store at room temperature
- Methanol
- Glacial acetic acid
- Purified water
- Rubber cement
- 12 N Hydrochloric acid (HCl)
- 1 N or 2 N Sodium hydroxide (NaOH)
- Formamide, Ultra Pure
- 0.075 M Potassium chloride (KCl)

Laboratory Equipment

- Glass microscope slides, precleaned
- 22 mm x 22 mm glass coverslips
- Microliter pipettor (1 to 10 µL) and sterile tips
- Timers
- Microcentrifuge
- Clinical centrifuge capable of reaching 250 x g and accommodating a 15 mL centrifuge tube
- 15 mL conical tubes
- Graduated cylinders (100 mL to 1000 mL)
- Water bath (70 to 80°C)
- Water bath (37°C)
- Forceps
- Disposable syringe
- Disposable pipettes (5 mL to 20 mL)
- Coplin jars/Vertical staining jar
- Fluorescence microscope equipped with recommended filters (see **Microscope Equipment and Accessories** section)
- Calibrated thermometer
- Microscope slide box with lid
- Magnetic stirrer
- pH meter
- pH indicator strips capable of reading pH 7.0 to 8.0
- Vortex mixer
- 0.45 µm filtration units
- 37°C incubator
- Humidified chamber
- Slide warmer (45 to 50°C)
- Cytogenetic drying chamber (optional)
- Microcentrifuge tubes
- Phase contrast microscope
- Oven capable of heating to 90°C or equivalent

Microscope Equipment and Accessories

Microscope An epi-illumination fluorescence microscope is required for viewing the hybridization results. The microscope should be checked to confirm it is operating properly to ensure optimum viewing of FISH assay specimens. A microscope used with general DNA stains such as DAPI, propidium iodide, and quinacrine may not function adequately for FISH assays. Routine microscope cleaning and periodic maintenance by the manufacturer's technical representative, especially alignment of the lamp, are advised.

Excitation Light Source A 100 watt mercury lamp or other lamp with similar intensity and spectral output is the recommended excitation source. The manufacturer's technical representative should be consulted to assure that the fluorescence illumination system is appropriate for viewing FISH assay specimens. Record the number of hours that the bulb has been used and replace the bulb before it exceeds the rated time. Ensure that the lamp is properly aligned.

Objectives Use oil immersion fluorescence objectives with numeric apertures ≥ 0.75 when using a microscope with a 100 watt mercury lamp or other lamp with similar intensity and spectral output. A 40X objective, in conjunction with 10X eyepieces, is suitable for scanning the specimen to select regions for enumeration. For enumeration of FISH signals, satisfactory results can be obtained with a 60/63X or 100X oil immersion achromat-type objective.

Immersion Oil The immersion oil used with oil immersion objectives should be one formulated for low auto fluorescence and specifically for use in fluorescence microscopy.

Filters Hybridization of the Vysis LSI TP53 SpectrumOrange, LSI ATM SpectrumGreen, LSI D13S319 SpectrumOrange, LSI 13q34 SpectrumAqua, and CEP 12 SpectrumGreen probes to their target regions of the DNA is marked by orange, aqua, and green fluorescence. The DNA which has not hybridized to the probes will fluoresce blue as a result of the DAPI II Counterstain.

The recommended filters for use with the Vysis CLL FISH Probe Kit are the Vysis Dual Band Green/Orange Filter V2, the Vysis Single Band DAPI Filter, the Vysis Single Band Orange Filter, the Vysis Single Band Aqua Filter, and the Vysis Single Band Green Filter or equivalents.

ASSAY PROTOCOL

Refer to the **WARNINGS AND PRECAUTIONS** section of this package insert before preparing samples.

Working Reagent Preparation

Ethanol Solutions (70%, 85%, and 100%)

Prepare v/v dilutions of 70% and 85% ethanol using 100% ethanol and purified water. Store the prepared solutions at room temperature in tightly capped containers. Discard stock solution after 6 months or sooner if solution appears cloudy or contaminated. Dilutions in Coplin jars may be used for up to one week unless evaporation occurs or the solution becomes diluted or cloudy due to excessive use.

20X SSC Solution

Dissolve 66 g of 20X SSC salt using 200 mL of purified water in a suitable vessel. Measure pH at room temperature using a pH meter and adjust pH to 5.3 ± 0.2 using 12 N HCl, if necessary. Transfer solution to a graduated cylinder and add purified water until a final volume of 250 mL is reached. Mix and filter the prepared solution through a 0.45 µm filtration unit. Store prepared stock solution at room temperature. Discard the stock solution after 6 months or sooner if solution appears cloudy or contaminated.

2X SSC Solution

Mix thoroughly 100 mL of 20X SSC with 850 mL purified water in a suitable vessel. Measure pH at room temperature using pH meter to verify pH is 7.0 ± 0.2. If necessary adjust the pH using 1N or 2 N sodium hydroxide. Add purified water to bring final volume of the solution to 1 L. Mix and filter through a 0.45 µm filtration unit. Store prepared stock solution at room temperature. Discard stock solution after 6 months or sooner if solution appears cloudy or contaminated. Discard solution that was used in the assay at the end of each day.

Denaturation Solution (70% formamide/ 2X SSC Solution)

Mix thoroughly 49 mL formamide, 7 mL 20X SSC Solution, and 14 mL purified water. Measure pH at room temperature using pH indicator strips to verify pH is 7.0 to 8.0. Store prepared stock solution at 2 to 8˚C. Verify pH is 7.0 to 8.0 using a pH indicator strip prior to each use. Discard solution after one week.

0.4X SSC / 0.3% NP-40 Wash Solution

Mix thoroughly 20 mL 20X SSC Solution and 950 mL purified water in a suitable vessel. Add 3 mL of NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH at room temperature using a pH meter and adjust pH to 7.4 ± 0.1 with 1 N or 2 N sodium hydroxide. Add purified water to bring final volume of the solution to 1 L. Mix and filter through a 0.45 µm filtration unit. Store prepared stock solution at room temperature. Discard stock solution after 6 months or sooner if solution appears cloudy or contaminated. Discard solution that was used in the assay at the end of each day.

2X SSC / 0.1% NP-40 Wash Solution

Using a suitable vessel, mix thoroughly 100 mL 20X SSC Solution with 850 mL purified water. Add 1 mL NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH at room temperature using a pH meter and adjust pH to 7.0 ± 0.2 with 1 N or 2 N sodium hydroxide. Add purified water to bring final volume to 1 L. Mix and filter through a 0.45 µm filtration unit. Store prepared stock solution at room temperature. Discard stock solution after 6 months or sooner if solution appears cloudy or contaminated. Discard solution that was used in the assay at the end of each day.

Specimen Collection and Transport

Peripheral blood collections should be performed according to the laboratory's institution guidelines. The AGT Cytogenetics Laboratory Manual contains recommendations for specimen collection and processing.[22] It states that it is acceptable to collect the peripheral blood specimen in a sodium heparin blood collection tube. One (1) mL of peripheral blood is required for performing the assay. Specimens can be processed immediately or shipped on cold packs and stored at 2 to 8˚C up to 96 hours prior to the start of sample preparation. Specimens should never be iced or frozen. Specimens that are clotted or not shipped as indicated should not be used.

Specimen Processing

NOTE: Fixative (3:1 or 2:1 methanol:glacial acetic acid) should be prepared fresh daily.

1. Add 1 mL of peripheral blood to a 15 mL conical centrifuge tube.
2. Slowly add 10 mL of 0.075 M KCl. Mix gently by inverting the tube several times. Optional: incubate tube(s) for 10 to 20 minutes at room temperature or 37˚C.
3. Slowly add 2 mL of fixative to the cell suspension. Mix gently by inverting the tube several times.
4. Centrifuge 8 to 12 minutes at 1000 to 1200 RPM (225 to 250 x g).
5. Remove and discard all but approximately 0.5 to 1.0 mL of the supernatant carefully without disturbing the cell pellet. Resuspend the cell pellet by gently agitating the tube.
6. Slowly add 10 mL fixative to the cell suspension. Mix by inverting the tube several times.
7. Centrifuge 8 to 12 minutes at 1000 to1200 RPM (225 to 250 x g).
8. Repeat the fixative wash (Steps 5 to 7) two more times or until the fixative is clear.
9. Resuspend the cell pellet in fixative. Adjust the volume of cell suspension to a density appropriate for slide preparation. If desired, prepare a test slide to verify the cell density.
10. Allow specimen to stand in fixative at room temperature (15 to 30˚C) for a minimum of 10 minutes before proceeding to Slide Preparation.
11. Proceed to Slide Preparation or, if necessary, fixed pellets can be stored at -20˚C (± 5˚C) for up to 24 months. If needed, particularly after a prolonged storage, wash pellets with fixative before slide preparation.

Slide Preparation

12. For optimal results, a cytogenetic drying chamber may be used. Set the unit to a temperature of 20 to 30˚C with a relative humidity of 30 to 50%. If a cytogenetic chamber is not available, other means of achieving the optimal temperature and humidity for target preparation may be used.
13. Label each slide appropriately.
14. Ensure the sample is mixed adequately before preparing the slide. Using a transfer pipette, expel 5 to 25 µL of cell suspension onto the left half of a pre-cleaned microscope slide and 5 to 25 µL of cell suspension onto the right half of the slide, making two hybridization areas. Allow the slide to dry.
15. Using a phase contrast microscope, examine the number of interphase nuclei per field, under low power (10X objective). A minimum of 100 cells per low power field is suggested for optimum assay results. Adjust the cell suspension to achieve the recommended number of interphase nuclei.

    NOTE: An optimal specimen will contain little to no debris and/or cytoplasm. Nuclei should appear dark, gray, flat, and non-refractile. Adjust temperature, humidity and volume to achieve optimal nuclei morphology.
16. Bake slides at 90˚C for 10 minutes (optional).

Specimen Target Preparation

NOTE: Initiate Manual Probe Denaturation procedure prior to completing Step 19 of this section to ensure the materials have adequate time to thaw.

17. Transfer Denaturation Solution and 2X SSC Solution to individual Coplin jars or remove previously prepared Denaturation Solution from 2 to 8˚C storage.
18. Transfer the Coplin jar(s) with Denaturation Solution into a water bath. Heat until the internal temperature of the Denaturation Solution is 73 ± 1˚C.
19. Transfer 2X SSC Solution Coplin jar(s) to a water bath. Warm to the internal temperature 37 ± 1˚C. Age specimen slides in 2X SSC Solution for 30 minutes at 37 ± 1˚C. Alternatively, slides can be aged for 2 minutes in 2X SSC Solution heated to 73˚C ± 1˚C.
    NOTE: Confirm solutions temperature using a calibrated thermometer. Immerse no more than 4 slides simultaneously in each Coplin jar.
20. Remove slides from the 2X SSC Solution and immediately transfer to Coplin jar(s) containing 70% ethanol for a minimum of 1 minute. Following 70% ethanol, transfer to 85% ethanol for a minimum of 1 minute, and then to 100% ethanol for a minimum of 1 minute.
21. Following dehydration in ethanol, immerse the specimen slides in the Denaturation Solution at 73 ± 1˚C for 5 minutes. Start timing when the last slide has been added to the Coplin jar.
    NOTE: To maintain the proper temperature in Denaturation Solution, denature 4 slides simultaneously. If there are less than 4 slides, add blank slides that are at room temperature to bring the total to 4.
22. Remove specimen slides from the Denaturation Solution and immediately transfer to the Coplin jar containing 70% ethanol for a minimum of 1 minute. Following 70% ethanol, transfer to 85% ethanol for a minimum of 1 minute, and then to 100% ethanol for a minimum of 1 minute.
    NOTE: Keep the specimen slides in 100% ethanol until ready to dry all slides and apply the probe mixture.

Probe Denaturation

NOTE: Probe mixtures are formulated at intended use concentrations and should not be diluted. Doing so may lead to erroneous assay results.

23. Remove the preformulated probe mixtures from storage and allow the reagents to reach room temperature.
24. Centrifuge probes for 2 to 3 seconds. Vortex and centrifuge the mixtures again briefly.
25. Place tubes at 73 ± 1˚C for 5 minutes.
26. Centrifuge tubes for 2 to 3 seconds.
27. Place tubes on 45 to 50˚C slide warmer until ready to apply probe to target specimen.
    NOTE: If specimen slides are ready when probes are denatured, apply probes immediately to the hybridization areas on the slide(s).

Hybridization

28. Remove specimen slides from the 100% ethanol.
29. Dry each slide by touching the bottom edge to a paper towel (or equivalent) and wiping the underside (the side that does not contain specimen).
30. Transfer slides to a 45 to 50˚C slide warmer for up to 2 minutes to evaporate remaining ethanol.
31. Using a microliter pipettor, apply 10 µL of the Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen probe to one target area and apply coverslip without introducing bubbles. Repeat procedure for Vysis LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen probe mixture.
32. Seal coverslips using a syringe filled with rubber cement.
33. Place slides in a pre-warmed humidified box, cover tightly, and incubate at 37˚C ± 1˚C for 6 to 24 hours. Alternatively, a 37˚C humidified incubator may be used.

Wash Procedure

34. Transfer a sufficient volume of 0.4X SSC/0.3% NP-40 and 2X SSC/0.1% NP-40 into individual Coplin jars to cover the slides. Transfer Coplin jar(s) containing 0.4X SSC/0.3% NP-40 to a water bath. Heat until the internal temperature of 0.4X SSC/0.3% NP-40 solution is 73 ± 1˚C. Confirm the solution temperature using a calibrated thermometer. Use 2X SSC/0.1% NP-40 at room temperature. Use solutions for 1 day only, and then discard.
35. Remove coverslips from one slide and immediately immerse the slide in the 0.4X SSC/0.3% NP-40. Agitate the specimen slides within Coplin jar for 1 to 3 seconds. Repeat process with other slides up to a total of 4 slides. Start timing when the fourth slide is immersed.
    NOTE: To maintain the proper temperature in 0.4X SSC/0.3% NP-40, wash 4 slides simultaneously. If there are less than 4 slides, add blank slides that are at room temperature to bring the total to 4.
36. Remove slides after 2 minutes ± 30 seconds.
37. Immerse slide(s) in Coplin jar containing 2X SSC/0.1% NP-40. Agitate the specimen slide(s) within Coplin jar for 1 to 3 seconds.
38. Remove slide(s) from 2X SSC/0.1% NP-40 after slides have been immersed for 5 to 60 seconds.

NOTE: If washing additional slides, ensure the temperature of the 0.4X SSC/0.3% NP-40 is 73 ± 1˚C before washing.

Counterstain Procedure

39. Dry each slide by touching the bottom edge to a paper towel (or equivalent) and wiping the underside (the side that does not contain specimen).
40. Air-dry the slides in darkness, at an angle, to facilitate evaporation and to prevent pooling of 2X SSC/0.1% NP-40, for a maximum of 2 hours.
41. Remove DAPI II Counterstain from storage and allow the reagent to reach room temperature.
42. Vortex DAPI II Counterstain for 2 to 3 seconds.
43. Centrifuge tube for 2 to 3 seconds.
44. Using a microliter pipettor, apply 10 µL of DAPI II counterstain to each specimen target area on the slide and apply coverslip. Repeat for each slide.
45. Allow a minimum of 10 minutes prior to microscopic observation.

Archiving Procedure

Store hybridized slides at –20˚C protected from light. Under these conditions, the slides can be stored for up to 3 weeks after the application of DAPI II counterstain without significant loss in fluorescence signal intensity.

Slide Examination

View slides using a suitable filter set on an optimally performing fluorescence microscope. The following optical filter sets will allow visualization of the fluorophores used in the hybridization.

Using this Vysis filter (or equivalent)…	Allows excitation and emission of…
Single Band Green	SpectrumGreen fluorophore
Single Band Aqua	SpectrumAqua fluorophore
Single Band Orange	SpectrumOrange fluorophore
Single DAPI	DAPI
Dual Band Green/Orange Filter v2	SpectrumOrange and SpectrumGreen fluorophores

Routine slide evaluation and enumeration is conducted using a Dual Band filter. For an individual nucleus, if specific probe signal(s) appear weak with a Dual Band filter, it is recommended to use a single band filter to assist in enumeration.

Interpretation and Result Reporting

Quality Control

The probes included in the Vysis CLL FISH Probe Kit serve as internal controls. The presence of at least one hybridization signal for the probes in each probe mix assures that the probes are functioning correctly under the assay conditions, with the exception of D13S319 nullisomy, when both copies of D13S319 target are deleted. The LSI 13q34 SpectrumAqua probe functions as a control for D13S319 deletion status, probe mixture discrimination and overall hybridization quality.

Assessing Target Adequacy

Evaluate target hybridization adequacy using the following criteria. If the criteria are not met, the specimen slide should not be evaluated.

- **Nuclear Morphology:** Borders of cell nuclei should generally be distinguishable and intact.
- **Background:** The background should appear dark or black and be relatively free of fluorescent particles or haziness.
- **Probe Signal Intensity**: The signals should be bright, compact, round or oval shapes, distinct and easily evaluable.

Confirmation of Probe Target

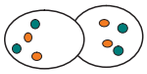
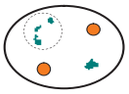
Immediately before signal enumeration of each hybridization area, use an epi-illumination fluorescence microscope with 60/63X or 100X objective and an aqua filter to determine and record the presence or absence of aqua signals in the hybridization area. This serves as a quality control measure to assure that the correct probe was applied to the hybridization area and that proper LSI-type hybridization occurred even when both copies of the Vysis LSI D13S319 Probe target are absent (no orange signals). An aqua signal will only be present on the Vysis LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen Probe hybridization site when appropriate hybridization conditions have occurred. There should be no aqua signals in the Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen Probe hybridization site.

Signal Enumeration

Using the appropriate filters listed above, two technologists or pathologists (readers) each determine and record the signal pattern present in each of 100 nuclei for both hybridization targets. The first reader scores the nuclei on half of the hybridization target (as best determined), and the second reader scores the nuclei on the other half of the hybridization target (as best determined). Refer to Table 1 for Dual Color Signal Counting Guide.

- Select only intact nuclei that are not folded, overlapped, or obstructed by debris.
- Avoid scoring slides or areas within slides that have excessive non-specific hybridization or many nuclei that have too few or no signals.
- Avoid scoring nuclei with clumps or clouds of signals.
- Signals of the same color that are touching, regardless of size, are counted as one signal. If there is a small strand of signal connecting separated signals, also count as one signal.
- In a signal area, if the signals of the same color have no gaps greater than a signal width, count as one signal. However, if two signals of the same color are distinct, compact, of the same size and intensity, are clearly separated, and no connecting signal strand is visible, count this as two signals even though the gap between signals is less than a signal width.
- Signals of the same color may not be the same intensity in a given nucleus. Therefore, it may be necessary to use the appropriate single pass filter and/or adjust the focal plane.
- If there is any doubt as to whether or not a cell should be scored, do not score the cell.

Table 1. Dual Color Signal Counting Guide

Key:		= Green = Orange
1		Do not count nuclei that are overlapping.
2		Count as two orange signals and two green signals. One orange signal is diffuse.
3		Count as two orange signals and two green signals. One orange signal is split.
4		Count as one orange signal and two green signals. One green signal is split and the orange signal is split.
5		Count as two orange signals and one green signal.
6		Signal area defined by dotted circle. No gaps in the upper signal area greater than an intact green signal width, the upper signal is counted as one green. Count as 2 orange and 2 green.
7		The two close orange signals are both compact and of the same intensity and size; count as two orange signals and two green signals.

After recording the results, the number of abnormal signal patterns of interest from each reader is examined and the following stepwise rules are applied to determine the final 200 nuclei count. Refer to Figure 1 for rules for combining scores and use of third reader and Table 2 and 3 for Cut-off Values for Inclusion of Third Reader for the Abnormal signal pattern.

Determining the Need for a Third Reader

The number of abnormal orange and green signal patterns from each reader are examined and compared against the Cut-off in Table 2 and Table 3.

Table 2. Normal Cut-off Values for Inclusion of Third Reader for Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen Probe

Probe/Color	Abnormal Pattern	Cut-off Value per 100 Nuclei	Abnormality
Vysis LSI TP53 SpectrumOrange	1 orange signal (1R)[a]	7	17p-
Vysis LSI ATM SpectrumGreen	1 green signal (1G)[b]	6	11q-

[a] The abnormal signal pattern for TP53 deletion (17p-) is 1R. Any pattern containing a single R signal, regardless of the number of G signals, is considered an abnormal pattern for TP53 deletion (17p-).

[b] The abnormal signal pattern for ATM deletion is 1G. Any pattern containing a single G signal, regardless of the number of R signals, is considered an abnormal pattern for ATM deletion.

Table 3. Normal Cut-off Values for Inclusion of Third Reader for Vysis LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen Probe

Probe/Color	Abnormal Pattern	Cut-off Value per 100 Nuclei[c]	Abnormality
Vysis LSI D13S319 SpectrumOrange	1 orange signal (1R)[a]	5.5	13q- (monosomy)
Vysis LSI D13S319 SpectrumOrange	zero orange signals (0R)[a]	1.5	13q- (nullisomy)
Vysis CEP 12 SpectrumGreen	3 green signals (3G) [b]	2.5	+12

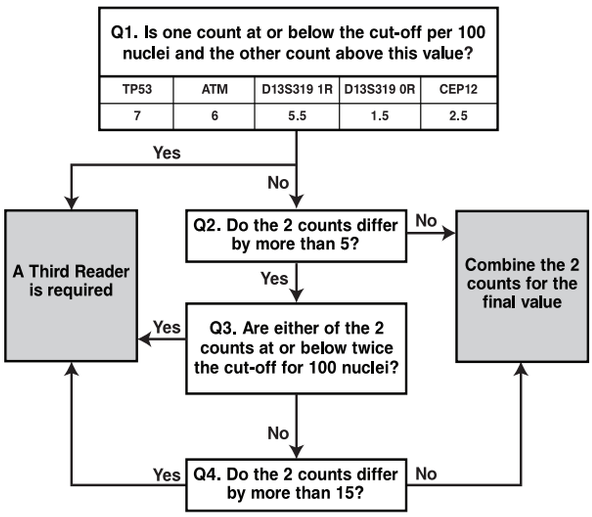
[a] The abnormal signal patterns for D13S319 deletion are 1R (monosomy) or 0R (nullisomy). Any pattern containing 1R or 0R signals, regardless of the number of G signals, is considered an abnormal pattern for D13S319 deletion.

[b] The abnormal signal pattern for CEP 12 gain is 3G (trisomy). Any pattern containing 3G signals, regardless of the number of R signals, is considered an abnormal pattern for CEP 12 gain.

[c] The value is half of the normal cut-off per 200 nuclei (see Tables 4 and 5).

Refer to Figure 1 for a description of the rules for determining the need for a third reader.

Figure 1. Rules for Combining Counts and Use of Third Reader for Each Abnormal Signal Pattern of Interest



For determining if a third reader is required, the counting rules are to be applied to each probe marker independently. The third reader counts are only used for the specific probe marker that requires a third reader. The Normal/Abnormal determination for the other probe markers is accomplished using the counts enumerated by the first two readers.

A third reader counts 100 nuclei in the hybridization target if any of the following apply:

- One reader has an abnormal signal pattern count at or below the cut-off for 100 nuclei and the other reader has a count above this value.
- The abnormal signal pattern counts determined by the two readers differ by more than 5 per 100 nuclei evaluated, and either of the two readers have abnormal signal pattern counts less than or equal to twice the cut-off value.
- The two readers have abnormal signal pattern counts which differ by more than 15 per 100 nuclei evaluated.

Results

The number of observed orange and green signal patterns, from each of two readers are added together to generate the count for 200 nuclei per specimen. When a third reader is used to complete the evaluation, the two scores closest to one another will be combined to generate the count for 200 nuclei. If any two scores are equidistant from the third score, the median value is doubled and used as the count for 200 nuclei.

Interpretation of Results

Normal/Abnormal Determination

Normal/Abnormal determination is made for each specimen by comparing the number of nuclei with observed abnormal patterns, per 200 scoreable nuclei, to a given normal cut-off value (Table 4 and Table 5). If the number of nuclei with a specific abnormal signal pattern exceeds its associated normal cut-off value, the result is abnormal. If the number of such nuclei is less than or equal to the normal cut-off value, the result is normal.

Table 4. Normal Cut-off Values for Determination of Normal/Abnormal for Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen Probe

Probe	Abnormal Pattern	Normal Cut-off Value per 200 Nuclei (%)	Abnormality
Vysis LSI TP53 SpectrumOrange	1 orange signal (1R)	14 (7.0%)	17p-
Vysis LSI ATM SpectrumGreen	1 green signal (1G)	12 (6.0%)	11q-

Table 5. Normal Cut-off Values for Determination of Normal/Abnormal for Vysis LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen Probe

Probe	Abnormal Pattern	Normal Cut-off Value per 200 Nuclei	Abnormality
Vysis LSI D13S319 SpectrumOrange	1 orange signal (1R)	11 (5.5%)	13q- (monosomy)
Vysis LSI D13S319 SpectrumOrange	zero orange signals (0R)	3 (1.5%)	13q- (nullisomy)
Vysis CEP 12 SpectrumGreen	3 green signals (3G)	5 (2.5%)	+12

Determination of Döhner Classification

Refer to **Table 6** to determine the **Döhner Classification** of each specimen based upon the abnormalities detected.[7]

Table 6. Döhner Classification
13q- (monosomy or nullisomy) as sole abnormality
No cytogenetic abnormality
+12 without 17p- or 11q-
11q- without 17p-
17p-

Prognostic Category Determination

Refer to Table 7 to determine the prognostic category (per Shanafelt et al.[10]) of each specimen based upon its Döhner Classification.

Table 7. Determination of Prognostic Category Based on Döhner Classification

Döhner Classification	Prognostic Category
13q- (monosomy or nullisomy) as sole abnormality	Good/Intermediate
No cytogenetic abnormality	Good/Intermediate
+12 without 17p- or 11q-	Good/Intermediate
11q- without 17p-	Poor
17p-	Poor

LIMITATIONS OF THE PROCEDURE

- FISH is a multiple step methodology that requires specialized training in the selection of the appropriate reagents, specimen preparation, processing, preparation of the FISH slide, and interpretation of the results.
- When used to select CLL patients for treatment with VENCLEXTA® (venetoclax), the test is validated for peripheral blood specimens anticoagulated with sodium heparin only.
- The Vysis CLL FISH Probe Kit is intended to be used in combination with additional biomarkers, morphology, and other clinical information.
- Other signal patterns may occur, and metaphase analysis may be helpful in characterization of such patterns.
- If a specimen has a low level abnormal FISH pattern, use of the appropriate single pass filter(s) to confirm the pattern is recommended. Failure to follow this recommendation may result in inaccurate identification of signal patterns.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Specificity

Analytical specificity is defined as the percentage of signals that hybridize to the correct locus and no other location. This test is a visual demonstration that each probe hybridizes specifically to the expected chromosome location. The analytical specificity of the probes in the Vysis CLL FISH Probe Kit for their respective chromosome target loci was established using metaphase chromosomes prepared from peripheral blood cultures of five karyotypically normal males on microscope slides. The hybridization location of each FISH signal on chromosomes of 20 consecutive metaphase nuclei on each of 5 slides was evaluated by one technologist for a total of 200 target loci.

For each probe and sample, the number of metaphase chromosome FISH signals hybridized to the correct locus and the number of metaphase chromosome FISH signals hybridized to the incorrect locus were enumerated. The specificity of each probe was calculated as the number of metaphase chromosome FISH signals hybridized to the correct locus divided by the total number of metaphase chromosome FISH signals hybridized and multiplied by 100 to give a percentage.

The analytical specificity for each probe of the Vysis CLL FISH Probe Kit was 100% (200/200) (refer to Table 8).

Table 8. Analytical Specificity

Probe	Correct Cytogenetic Target	No. of Metaphase Chromosome Signals: Hybridized to the Correct Target Locus	No. of Metaphase Chromosome Signals: Total Hybridized Signals	Specificity (%)	95% Confidence Interval (%)
Vysis LSI TP53 SpectrumOrange	17p13.1	200	200	100	(98.17, 100)
Vysis LSI ATM SpectrumGreen	11q22.3	200	200	100	(98.17, 100)
Vysis LSI D13S319 SpectrumOrange	13q14.3	200	200	100	(98.17, 100)
Vysis LSI 13q34 SpectrumAqua	13q34	200	200	100	(98.17, 100)
Vysis CEP 12 SpectrumGreen	12p11.1-q11	200	200	100	(98.17, 100)

Analytical Sensitivity

Analytical sensitivity is defined as the percentage of scoreable interphase nuclei with the expected normal signal pattern. The expected normal interphase signal pattern for all probes in the Vysis CLL FISH Probe Kit is 2 signals per nucleus.

The analytical sensitivity of the probes in the Vysis CLL FISH Probe Kit for their respective chromosome target loci was established using interphase nuclei prepared from peripheral blood cultures of 25 karyotypically normal patients. For each specimen, the signal patterns of 200 nuclei were evaluated by counting the number of orange and green signals present for each probe target. Each technologist evaluated 100 nuclei per specimen for a total of 200 nuclei per specimen and 5000 scoreable nuclei from normal specimens.

The sensitivity (with 95% confidence intervals based on binomial distribution) was calculated as the percentage of scoreable interphase nuclei with the expected signal pattern of 2 signals per nucleus.

Table 9 shows that the Vysis CLL FISH Probe Kit has a sensitivity of 97.98% for the LSI TP53 SpectrumOrange probe, 98.68% for the LSI ATM SpectrumGreen probe, 98.60% for the LSI D13S319 SpectrumOrange probe, and 98.94% for the CEP 12 SpectrumGreen probe.

Table 9. Analytical Sensitivity

Probe	Number of Interphase Nuclei: With Expected Signal Pattern	Number of Interphase Nuclei: Scoreable Signals	Sensitivity: Percent (%)	Sensitivity: 95% Confidence Interval (%)
Vysis LSI TP53 SpectrumOrange	4899	5000	97.98	97.55, 98.35
Vysis LSI ATM SpectrumGreen	4934	5000	98.68	98.32, 98.98
Vysis LSI D13S319 SpectrumOrange	4930	5000	98.60	98.23, 98.91
Vysis CEP 12 SpectrumGreen	4947	5000	98.94	98.62, 99.21

Analytical Characterization of Normal Cut-off Values

The normal cut-off value, in association with FISH DNA probes, is defined as the maximum percentage of scoreable interphase nuclei with a specific abnormal signal pattern at which a specimen is considered normal for that signal pattern. The normal cut-off value is expressed in terms of a percentage or the actual number of abnormal nuclear FISH patterns per the standard number of nuclei tested. The criteria used to classify a nucleus as scoreable is located in the **Interpretation and Result Reporting** section of this package insert. The **Quality Control** and **Signal Enumeration** sections and the **Dual Color Signal Counting Guide (Table 1)** instruct the end user to determine whether an entire slide is adequate for signal enumeration and which types of cells/signals can be enumerated.

The normal cut-off values of the probes in the Vysis CLL FISH Probe Kit for their respective chromosome target loci were established using interphase nuclei prepared from peripheral blood cultures of 25 karyotypically normal patients. For each specimen, the signal patterns of 200 nuclei were evaluated by counting the number of orange and green signals present for each probe target.

The expected normal interphase signal pattern for all probes in the Vysis CLL FISH Probe Kit was two signals per nucleus. Since the specimen population does not fit a Gaussian distribution, the normal cut-off value was calculated using the beta inverse function.[23]

The Vysis CLL FISH Probe Kit was shown to have normal cut-off values of 14 out of 200 nuclei evaluated (7.0%) for the LSI TP53 SpectrumOrange probe (1 signal), 12 out of 200 nuclei evaluated (6.0%) for the LSI ATM SpectrumGreen probe (1 signal), 11 out of 200 nuclei evaluated (5.5%) for the LSI D13S319 SpectrumOrange probe (1 signal), 3 out of 200 nuclei evaluated (1.5%) for the LSI D13S319 SpectrumOrange probe (0 signal), and 5 out of 200 nuclei evaluated (2.5%) for the CEP 12 SpectrumGreen probe (3 signals). Refer to Table 10.

Table 10. Analytical Characterization of Normal Cut-off Values

Probe (Abnormal Signal Pattern of Interest)	No. of Nuclei Evaluated (n)	Maximum No. of False Positive Patterns	Normal Cut-off Value (per 200 Nuclei)	Normal Cut-Off Value (%)
Vysis LSI TP53 SpectrumOrange (1 signal)	200	8	14	7.0 (14/200)
Vysis LSI ATM SpectrumGreen (1 signal)	200	6	12	6.0 (12/200)
Vysis LSI D13S319 SpectrumOrange (1 signal)	200	5	11	5.5 (11/200)
Vysis LSI D13S319 SpectrumOrange (0 signal)	200	0	3	1.5 (3/200)
Vysis CEP 12 SpectrumGreen (3 signals)	200	1	5	2.5 (5/200)

Precision

The precision of the probes in the Vysis CLL Probe Kit was established using interphase nuclei prepared from 2 separate peripheral blood specimens lacking del (17p13.1), del (11q22.3), del (13q14.3), and trisomy 12, and 8 additional specimens of which at least 2 specimens had one of the listed abnormalities (Precision Study 1).

This blinded 10-member slide panel consisting of both negative and positive specimens was used to test 3 lots of Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen and Vysis LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen Probes on each of 2 days, and 1 of the 3 lots on a third day.

For each specimen, the FISH signal patterns of 200 nuclei were evaluated by counting the number of orange and green signals present for each probe target.

Precision was analyzed separately for each specimen per probe using the percentage of abnormal cells with the signal pattern(s) of interest.

A blinded panel created from 8 unique specimens was tested using three different DNA FISH probe lots on 3 days (Precision Study 2). Precision analyses to challenge the normal cut-off values were performed using the percentage of cells with the signal pattern of interest and were analyzed separately for each panel number.

Precision Study 1 and Precision Study 2 mean and standard deviations of the observed percentages of abnormal cells of the negative, positive, and specimens near the normal cut-off are shown in **Tables 11** through **15**.

Table 11. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis LSI TP53 SpectrumOrange Probe [del(17p13.1)(1 signal)][a]

Study	Sample	Category	n	Mean	Between-Day (Within Lot) Component SD	Between-Lot Component SD	Total SD[b]
1	1	Negative	7	2.6	1.23	0.00	1.23
1	2	Negative	7	2.9	1.58	1.11	1.94
1	3	Negative	7	3.8	3.48	0.00	3.48
1	4	Negative	7	3.1	2.00	0.00	2.00
1	5	Negative	7	2.7	0.76	0.65	1.00
1	6	Negative	7	2.4	2.90	0.00	2.90
1	7	Negative	7	2.1	1.38	0.00	1.38
1	8	Negative	7	2.8	1.29	0.00	1.29
1	9	Positive	7	29.8	5.15	4.54	6.87
1	10	Positive	7	73.2	5.29	0.00	5.29
2	7	Positive[c]	9	13.6	3.11	0.00	3.11
2	8	Positive[c]	9	16.9	3.90	0.00	3.90

a The mean and standard deviations are represented as percentages of abnormal signal patterns.

b Total variance is the sum of the other variance components.

c Positive specimen near the normal cut-off.

Table 12. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis LSI ATM SpectrumGreen Probe [del(11q22.3)(1 signal)][a]

Study	Sample	Category	n	Mean	Between-Day (Within Lot) Component SD	Between-Lot Component SD	Total SD[b]
1	1	Negative	7	1.6	0.54	0.59	0.80
1	2	Negative	7	1.8	0.59	0.00	0.59
1	3	Negative	7	2.6	0.81	1.54	1.74
1	4	Negative	7	2.1	2.25	1.08	2.50
1	5	Negative	7	2.9	1.71	1.64	2.37
1	6	Negative	7	1.1	1.34	0.00	1.34
1	7	Positive	7	64.1	3.18	0.00	3.18
1	8	Positive	7	13.7	4.31	5.10	6.68
1	9	Negative	7	1.6	0.99	0.00	0.99
1	10	Negative	7	1.5	1.51	0.00	1.51
2	1	Positive[c]	9	8.4	3.12	0.91	3.25
2	2	Positive[c]	9	7.8	3.00	1.89	3.55
2	8	Positive[c]	9	18.7	3.46	0.00	3.46

a The mean and standard deviations are represented as percentages of abnormal signal patterns.

b Total variance is the sum of the other variance components.

c Positive specimen near the normal cut-off.

Table 13. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis LSI D13S319 SpectrumOrange Probe [del(13q14.3)(1 signal)][a]

Study	Sample	Category	n	Mean	Between-Day (Within Lot) Component SD	Between-Lot Component SD	Total SD[b]
1	1	Negative	7	3.5	1.12	1.10	1.57
1	2	Negative	7	2.2	1.69	0.00	1.69
1	3	Positive	7	66.1	6.13	0.00	6.13
1	4	Positive	7	20.1	4.88	0.00	4.88
1	5	Negative	7	0.6	0.83	0.00	0.83
1	6	Negative	7	0.4	0.64	0.00	0.64
1	7	Positive	7	84.9	4.14	0.00	4.14
1	8	Negative	7	0.6	0.97	0.00	0.97
1	9	Negative	7	2.3	2.08	0.00	2.08
1	10	Positive	7	53.6	8.57	0.00	8.57
2	5	Positive[c]	9	5.8	2.49	0.00	2.49
2	6	Positive[c]	9	14.9	2.46	1.15	2.72

a The mean and standard deviations are represented as percentages of abnormal signal patterns.
b Total variance is the sum of the other variance components.
c Positive specimen near the normal cut-off.

Table 14. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis LSI D13S319 SpectrumOrange Probe [del(13q14.3)(0 signals)][a]

Study	Sample	Category	n	Mean	Between-Day (Within Lot) Component SD	Between-Lot Component SD	Total SD[b]
1	1	Negative	7	0.0	0.00	0.00	0.00
1	2	Negative	7	0.0	0.00	0.00	0.00
1	3	Negative	7	0.2	0.21	0.38	0.44
1	4	Negative	7	0.1	0.40	0.00	0.40
1	5	Negative	7	0.0	0.00	0.00	0.00
1	6	Negative	7	0.0	0.00	0.00	0.00
1	7	Negative	7	0.2	0.32	0.00	0.32
1	8	Negative	7	0.0	0.00	0.00	0.00
1	9	Negative	7	0.0	0.00	0.00	0.00
1	10	Positive	7	18.4	6.32	0.87	6.38
2	8	Positive[c]	9	19.8	2.53	0.00	2.53

a The mean and standard deviations are represented as percentages of abnormal signal patterns.
b Total variance is the sum of the other variance components.
c Positive specimen near the normal cut-off.

Table 15. Precision Analysis of Percentages of Abnormal Signal Patterns for Vysis CEP 12 SpectrumGreen Probe [trisomy 12 (3 signals)][a]

Study	Sample	Category	n	Mean	Between-Day (Within Lot) Component SD	Between-Lot Component SD	Total SD[b]
1	1	Negative	7	0.2	0.42	0.00	0.42
1	2	Negative	7	0.2	0.60	0.00	0.60
1	3	Negative	7	0.0	0.00	0.00	0.00
1	4	Negative	7	0.1	0.40	0.00	0.40
1	5	Positive	7	21.6	6.07	0.00	6.07
1	6	Positive	7	70.7	4.06	1.07	4.19
1	7	Negative	7	0.0	0.00	0.00	0.00
1	8	Positive	7	67.3	4.84	0.00	4.84
1	9	Negative	7	0.0	0.00	0.00	0.00
1	10	Negative	7	0.1	0.20	0.00	0.20
2	3	Positive[c]	9	8.3	2.77	0.00	2.77
2	4	Positive[c]	9	3.6	3.05	0.00	3.05

a The mean and standard deviations are represented as percentages of abnormal signal patterns.
b Total variance is the sum of the other variance components.
c Positive specimen near the normal cut-off.

Reproducibility I

Three individual laboratories tested a blinded 20-member slide panel consisting of specimens representing the five Döhner classifications (refer to **Table 7**). The analysis was conducted with 10 slides per day for 2 days resulting in a total of 6 runs across 3 testing sites. The same lots of Vysis LSI TP53 SpectrumOrange/ATM SpectrumGreen and LSI D13S319 SpectrumOrange/13q34 SpectrumAqua/CEP 12 SpectrumGreen Probes were tested at each laboratory. Results shown in **Table 16** show an overall agreement with specimens representing each of the 5 Döhner classifications.

Table 16. Overall Agreement, Site to Site by Probe

Probe (Abnormality)	Disagree[a] Site 1	Disagree[a] Site 2	Disagree[a] Site 3	Overall Number Agree[a]	Overall Number Tested	Overall Percent Agreement
TP53 (17p-)	0	0	0	20	20	100
ATM (11q-)	2	0	0	18	20	90
CEP 12 (+12)	0	0	0	20	20	100
D13S319 1x (13q-)	2	0	0	18	20	90
D13S319 2x (13q-)	0	1	1	18	20	90

[a] Disagree = Number of specimens for which one's site result did not agree with the other sites' results. Agree = Number of specimens for which all 3 sites agreed on results.

The reproducibility of the Vysis CLL FISH Probe Kit for each Prognostic Category demonstrated almost perfect agreement[24] using a generalized kappa statistic among the three testing sites (**Table 17**).

Table 17. Vysis CLL FISH Probe Kit Reproducibility Based on Generalized Kappa Statistic

Sample	Prognostic Category: Good/Intermediate Prognosis[a]	Prognostic Category: Poor Prognosis[a]
1	3	0
2	3	0
3	3	0
4	3	0
5	3	0
6	3	0
7	3	0
8	3	0
9	3	0
10	3	0
11	3	0
12	3	0
13	0	3
14	1	2
15	0	3
16	1	2
17	0	3
18	0	3
19	0	3
20	0	3
Kappa	0.86	
Strength	Almost Perfect[24]	

a Number of sites based on Prognostic Category

Additionally, the data were analyzed by site and probe using the Fisher's Exact Test. Refer to **Tables 18** and **19**.

Table 18. Vysis CLL FISH Probe Kit Reproducibility by Site (Based on Fisher's Exact Test)

	Prognostic Category	
Site	Good/Intermediate[a]	Poor[a]
1	12	8
2	12	8
3	14	6
p-value	0.8396	

a Number of panel members based on Prognostic Category.

Table 19. Vysis CLL FISH Probe Kit Reproducibility by Probe Based on Fisher's Exact Test

Probe (Abnormality)	Site	Abnormality Detected	No Abnormality Detected	*p*-value
TP53 (17p-)	1	4	16	1.0000
	2	4	16	
	3	4	16	
ATM (11q-)	1	2	18	0.7495
	2	4	16	
	3	4	16	
D13S319 1x (13q-[x1])	1	6	14	0.8396
	2	8	12	
	3	8	12	
D13S319 2x (13q-[x2])	1	2	18	1.000
	2	3	17	
	3	3	17	
CEP 12 (+12)	1	5	15	1.000
	2	5	15	
	3	5	15	

Reproducibility II -17p Deletion

Reproducibility of the Vysis CLL FISH Probe Kit was evaluated at 3 external laboratories by testing a coded, randomized 8-member specimen panel that consisted of 6 unique 17p deletion positive specimens with varying target levels of positivity (near-cutoff 1 and 2, low positive 1 and 2, high positive 1 and 2) and 2 unique 17p deletion negative peripheral blood specimens (normal 1 and 2).

The 8-member panel consisted of 2 panel members of each of the following:

Specimen Type	Target Percent Positive Cells
Normal	< 7%
Near cutoff positive (17p-)	7% to 19%
Low positive (17p-)	20% to 50%
High positive (17p-)	> 50%

The 8 panel members averaged to the following 17p deletion levels across 90 replicates: Normal 1 (3.5%), Normal 2 (3.4%), Near Cutoff 1 (12.9%), Near Cutoff 2 (13.3%), Low Positive 1 (52.6%), Low Positive 2 (20.8%), High Positive 1 (63.5%), High Positive 2 (82.1%).

Three lots of the Vysis CLL FISH Probe Kit reagents were used for the evaluation at each of the 3 sites.

A minimum of 2 dedicated technologists at each of the 3 testing sites enumerated the panel members for 17p deletion. Testing for each reagent lot was for 5 non-consecutive days over a time period of approximately 25 calendar days.

Each site evaluated 240 specimen slides for a total of 720 (8 specimens × 2 replicates per day × 5 days × 3 lots × 3 sites). For each panel member slide, the signal patterns of 100 nuclei were enumerated by two readers for a combined total of 200 nuclei.

Lot-to-lot and site-to-site pairwise comparisons were conducted. Refer to Table 20 and Table 21.

The Overall Agreement (OA), Average Positive Agreement (APA), and Average Negative Agreement (ANA) for 17p deletion results were calculated from all possible pairwise comparisons for each panel member for the between-lot and for the between-site agreement. The corresponding 95% bootstrap confidence intervals were calculated as the 2.5 and 97.5 percentiles from 1,000 pairwise agreements.

For panel members with 100% agreement, a score confidence interval was calculated. This score confidence interval was calculated using 200 out of 300 pairs, since the remaining pairs can be mapped from the 200 pairs that were used.

For the lot-to-lot comparison the pairs consisted of lot 1 vs. lot 2, lot 1 vs. lot 3, and lot 2 vs. lot 3. For lot A vs. lot B comparison, lot A refers to the first lot in the pairs and lot B refers to the second lot in the pairs. Refer to Table 20 for the lot-to-lot pairwise comparisons.

A similar analysis was done for the site-to-site comparison but with the pairs consisting of site 1 vs. site 2, site 1 vs. site 3, and site 2 vs. site 3. Refer to Table 21 for the site-to-site pairwise comparisons.

Table 20. 17p Deletion: Reproducibility Lot-to-Lot Pairwise Comparisons

			First Lot Result Second Lot Result				Point Estimate and 95% CI		
Panel	Description	n	Abnormal Abnormal	Normal Normal	Abnormal Normal	Normal Abnormal	ANA	APA	OA
Normal 1	Lot 1, 2	300	0	290	10	0	98% (95%,100%)	N/A	97% (90%,100%)
	Lot 1, 3	300	3	263	7	27	94% (89%,98%)	15% (0%,30%)	89% (80%,97%)
	Lot 2, 3	300	0	270	0	30	95% (89%,99%)	N/A	90% (80%,98%)
	Lot A vs. B	900	3	823	17	57	96% (92%,99%)	8% (0%,15%)	92% (85%,98%)
Normal 2	Lot 1, 2	300	1	281	9	9	97% (92%,100%)	10% (0%,24%)	94% (86%,100%)
	Lot 1, 3	300	1	281	9	9	97% (93%,100%)	10% (0%,24%)	94% (87%,100%)
	Lot 2, 3	300	1	281	9	9	97% (93%,100%)	10% (0%,20%)	94% (87%,100%)
	Lot A vs. B	900	3	843	27	27	97% (94%,100%)	10% (0%,18%)	94% (88%,100%)
Near Cutoff 1	Lot 1, 2	300	253	3	27	17	12% (0%,29%)	92% (85%,98%)	85% (75%,97%)
	Lot 1, 3	300	280	0	0	20	N/A	97% (91%,100%)	93% (83%,100%)
	Lot 2, 3	300	270	0	0	30	N/A	95% (89%,100%)	90% (80%,100%)
	Lot A vs. B	900	803	3	27	67	6% (0%,14%)	94% (90%,99%)	90% (82%,98%)
Near Cutoff 2	Lot 1, 2	300	280	0	10	10	N/A	97% (91%,100%)	93% (83%,100%)
	Lot 1, 3	300	290	0	0	10	N/A	98% (95%,100%)	97% (90%,100%)
	Lot 2, 3	300	290	0	0	10	N/A	98% (95%,100%)	97% (90%,100%)
	Lot A vs. B	900	860	0	10	30	N/A	98% (94%,100%)	96% (89%,100%)
Low Positive 1*	Lot 1, 2	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot 1, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot 2, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%, 100%)
	Lot A vs. B	900	900	0	0	0	N/A	100% (99%,100%)	100% (99%,100%)
Low Positive 2	Lot 1, 2	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot 1, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot 2, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot A vs. B	900	900	0	0	0	N/A	100% (99%,100%)	100% (99%,100%)
High Positive 1	Lot 1, 2	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot 1, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot 2, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot A vs. B	900	900	0	0	0	N/A	100% (99%,100%)	100% (99%,100%)
High Positive 2	Lot 1, 2	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot 1, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot 2, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Lot A vs. B	900	900	0	0	0	N/A	100% (99%,100%)	100% (99%,100%)

N/A = not applicable; no observations in these categories

* Low Positive 1 was initially measured at 40% positive cells at the screening stage. Upon analysis of reproducibility study data, the mean percentage of positive cells was measured to 52.6%.

Table 21. 17p Deletion: Reproducibility Site-to-Site Pairwise Comparisons

			First Site Result Second Site Result				Point Estimate and 95% CI		
Panel	Description	n	Abnormal Abnormal	Normal Normal	Abnormal Normal	Normal Abnormal	ANA	APA	OA
Normal 1	Site 1, 2	300	0	260	0	40	93% (85%,98%)	N/A	87% (73%,97%)
	Site 1, 3	300	0	300	0	0	100% (98%,100%)	N/A	100% (98%,100%)
	Site 2, 3	300	0	260	40	0	93% (85%,98%)	N/A	87% (73%,97%)
	Site A vs. B	900	0	820	40	40	95% (90%,99%)	N/A	91% (82%,98%)
Normal 2	Site 1, 2	300	0	270	0	30	95% (87%,100%)	N/A	90% (77%,100%)
	Site 1, 3	300	0	300	0	0	100% (98%,100%)	N/A	100% (98%,100%)
	Site 2, 3	300	0	270	30	0	95% (87%,100%)	N/A	90% (77%,100%)
	Site A vs. B	900	0	840	30	30	97% (92%,100%)	N/A	93% (84%,100%)
Near Cutoff 1	Site 1, 2	300	290	0	10	0	N/A	98% (95%,100%)	97% (90%,100%)
	Site 1, 3	300	260	0	40	0	N/A	93% (85%,98%)	87% (73%,97%)
	Site 2, 3	300	251	1	39	9	4% (0%,15%)	91% (82%,97%)	84% (70%,93%)
	Site A vs. B	900	801	1	89	9	2% (0%,8%)	94% (89%,98%)	89% (80%,96%)
Near Cutoff 2	Site 1, 2	300	290	0	10	0	N/A	98% (95%,100%)	97% (90%,100%)
	Site 1, 3	300	290	0	10	0	N/A	98% (95%,100%)	97% (90%,100%)
	Site 2, 3	300	280	0	10	10	N/A	97% (91%,100%)	93% (83%,100%)
	Site A vs. B	900	860	0	30	10	N/A	98% (94%,100%)	96% (89%,100%)
Low Positive 1*	Site 1, 2	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site 1, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site 2, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site A vs. B	900	900	0	0	0	N/A	100% (99%,100%)	100% (99%,100%)
Low Positive 2	Site 1, 2	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site 1, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site 2, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site A vs. B	900	900	0	0	0	N/A	100% (99%,100%)	100% (99%,100%)
High Positive 1	Site 1, 2	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site 1, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site 2, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site A vs. B	900	900	0	0	0	N/A	100% (99%,100%)	100% (99%,100%)
High Positive 2	Site 1, 2	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site 1, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site 2, 3	300	300	0	0	0	N/A	100% (98%,100%)	100% (98%,100%)
	Site A vs. B	900	900	0	0	0	N/A	100% (99%,100%)	100% (99%,100%)

N/A = not applicable; no observations in these categories

* Low Positive 1 was initially measured at 40% positive cells at the screening stage. Upon analysis of reproducibility study data, the mean percentage of positive cells was measured to 52.6%.

Clinical Utility

The traditional Rai and Binet clinical CLL staging systems are based on disease burden and have been useful for assigning patients to groups having similar survival times.[25,26] These systems, however, are not effective in predicting survival in early-stage disease when most CLL cases are diagnosed. This has led to development of molecular markers that differentiate those patients who are prone to rapid progression from those who have indolent disease.

In a pivotal study conducted by Döhner et al, titled "Genomic Aberrations and Survival in Chronic Lymphocytic Leukemia," genomic alterations as determined by FISH were found to be predictive for disease progression and overall survival.[7] Multiple studies support the conclusion of Döhner et al. that loss of 17p and of 11q markers predicts reduced survival times as compared to other Döhner groups as determined by FISH aberrations.[8-10] Such studies have led to the inclusion of FISH testing in the NCCN practice guidelines as a means to determine CLL prognosis. CLL prognostic categories according to the current NCCN guidelines divide patients into 3 categories – favorable, neutral and unfavorable.[11]

In a 2006 prospective study of 151 patients by Shanafelt et al utilizing Vysis FISH probes, a correlation was established between overall survival and FISH risk category for CLL at diagnosis.[10] Patients were divided into 2 prognostic groups. They were assigned to the good/intermediate FISH prognosis group if there were no chromosomal aberrations or if only 13q- and/or +12 aberrations were present. If a chromosomal aberration of 17p- or 11q- was present, the patient was placed in the poor FISH prognosis group. Poor vs. good/intermediate FISH (P=0.004), age at diagnosis (P=0.0006); and Rai stage (P=0.0026) were each significantly associated with overall survival from diagnosis in univariate analysis. When all factors were included in multivariable Cox regression model, each of three factors still remained significant: Poor vs. good/intermediate FISH (P=0.00022), age at diagnosis (P=0.000024); and Rai stage (P=0.00012).[10]

The clinical utility of the Vysis CLL FISH Probe Kit has been established primarily from its high concordance with the assay employed in the publication by Shanafelt et al.[10] (See **Method Concordance.**)

Also, as noted in the Shanafelt study, all patients with the 17p- abnormality had between 24 to 94% of cells with this abnormality.[10] Therefore, the effect of 17p- at very low levels could not be determined. In a publication on untreated 17p- CLL patients, Tam et al reported a 3-year overall survival of 92% for patients with < 25% 17p-deleted nuclei, vs. 54% for patients with ≥ 25% 17p-deleted nuclei (P=0.007).[15]

Method Concordance

A study was done comparing the method used in the Vysis CLL FISH Probe Kit and the method used by Shanafelt et al.[10] This study established the clinical validity of the Vysis CLL FISH Probe Kit by demonstrating concordance to the FISH method used in the Shanafelt study. The clinical validity of the Vysis CLL FISH Probe Kit is documented via peer-reviewed literature.

The study analyzed 64 specimens whose Döhner classifications were based on previous results using the Shanafelt FISH method. Table 22 shows the distribution of specimens analyzed, by Döhner classification.

Table 22. Number of Specimens Analyzed, According to Döhner Classification

Döhner Classification	Number of Specimens Analyzed
13q- (monosomy or nullisomy) as sole abnormality	13
Normal (no cytogenetic abnormalities)	12
+12 without 11q- or 17p-	14
11q- without 17p-	18
17p-	7

A Döhner classification was assigned to each result (Table 6), and the Prognostic Category was determined (refer to Table 7).

The percent agreement between the Vysis CLL FISH Probe Kit and Shanafelt FISH method for Prognostic Category was 97% (62/64) with a lower bound of the one-sided 95% confidence interval of 90%. Overall agreement was defined as the percentage of specimens that had the same Prognostic Category when tested using the Vysis CLL FISH Probe Kit and the Shanafelt FISH method (refer to Table 23).

Table 23. Concordance for Prognostic Category[a]

	Reference Shanafelt FISH Test		
Vysis CLL FISH Probe Kit	**Good/ Intermediate**	**Poor**	**Total**
Good/Intermediate	38	1	39
Poor	1	24	25
Total	39	25	64

a The values in this table represent the number of specimens

Clinical Trial Information

The clinical trial was a multi-center study testing peripheral blood specimens from relapsed or refractory CLL subjects. The efficacy of VENCLEXTA® (venetoclax) (a BCL2 inhibitor) was investigated in a single-arm trial of previously treated CLL patients with 17p deletion (Study 1, M13-982 trial). Study 1 was performed with the Vysis CLL FISH Probe Kit on peripheral blood specimens from relapsed/refractory CLL patients. A total of 167 subjects were screened for 17p deletion in the Study 1 main cohort.

Of the 167 subjects screened, 106 were enrolled in Study 1. Demographic and disease characteristics of the study population for Study 1 are provided in Table 24.

Table 24. Demographic and Disease Characteristics in Study 1

Characteristics		**N=106**	
Age (Years)			
	Median (Range)	67	(37-83)
Race, n (%)			
	Asian	0	(0)
	Black	3	(2.8)
	White/Unknown[a]	103	(97.2)
Gender, n (%)			
	Male	69	(65.1)
	Female	37	(34.9)
ECOG, n (%)			
	0	42	(39.6)
	1	55	(51.9)
	2	9	(8.5)
Number of Prior Therapies			
	Median (Range)	2.5	(1-10)

[a] Race unknown for one patient

In this study, 17p deletion status was determined using the Vysis CLL FISH Probe Kit. Efficacy data from Study 1 are provided in Table 25. The primary efficacy endpoint was overall response rate (ORR) as assessed by an Independent Review Committee (IRC) using the International Workshop for Chronic Lymphocytic Leukemia (IWCLL) updated Nation Cancer Institute-sponsored Working Group (NCI-WG) guidelines (2008).[27]

Table 25. Efficacy Results for Patients with Previously Treated CLL with 17p deletion by IRC (Study 1)

	VENCLEXTA® N (%) N=106
ORR, n (%)	85 (80.2)
(95% CI)	(71.3, 87.3)
CR + CRi, n (%)	8 (7.5)
CR, n (%)	6 (5.7)
CRi, n (%)	2 (1.9)
nPR, n (%)	3 (2.8)
PR, n (%)	74 (69.8)

CI = confidence interval; CR = complete remission; CRi = complete remission with incomplete marrow recovery; IRC = Independent review committee; nPR = nodular partial remission; ORR = overall response rate (CR + CRi + nPR + PR); PR = partial remission.

R/R CLL patients with 17p deletion were evaluated for the efficacy of VENCLEXTA® (venetoclax) by overall response rate (ORR). 80.2% ORR was observed. Refer to the drug label for more information.

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