CEP X SpectrumOrange/ Y SpectrumGreen DNA Probe Kit en

REF 07J20-050
07J22-050

30-608310/R3

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NOTE: Changes Highlighted

Key to Symbols Used

	Legal Manufacturer
REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
	Contains sufficient for <n> tests
	Temperature limitation
	Danger
	Danger
	Danger
	Biological Risks
	Consult instructions for use
	Used by
EC REP	Authorized Representative in the European Community

CUSTOMER SERVICE: 1-800-553-7042

CUSTOMER SERVICE INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CEP X SPECTRUMORANGE/Y SPECTRUMGREEN DNA PROBE KIT (CEP X/Y)

(Part No. 30-161050, List No. 07J20-050)
(Part No. 32-161050, List No. 07J22-050)

PROPRIETARY NAME

CEP X SpectrumOrange/Y SpectrumGreen Direct Labeled Fluorescent DNA Probe Kit

COMMON OR USUAL NAME

Fluorescence in situ hybridization (FISH) reagents

INTENDED USE

The CEP X SpectrumOrange/Y SpectrumGreen DNA Probe Kit is intended to detect alpha satellite sequences in the centromere region of chromosome X and satellite III DNA at the Yq12 region of chromosome Y in conjunction with routine diagnostic cytogenetic testing. It is indicated for use as an adjunct to standard cytogenetic analysis for identifying and enumerating chromosomes X and Y via FISH in interphase nuclei and metaphase spreads obtained from bone marrow specimens in subjects who received opposite-sex bone marrow transplantation for chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), myeloproliferative disorder (MPD), myelodysplastic syndrome (MDS), acute and lymphoid leukemia (ALL), or hematological disorder not otherwise specified (HDNOS). It is not intended to be used as a stand alone assay for test reporting; FISH results are intended to be reported and interpreted only in conjunction with results from standard cytogenetic analysis, performed concurrently, using the same patient specimen. This device is not intended for use in subjects with like-sex bone marrow transplants; with matrices other than unstimulated, cultured bone marrow specimens; or in screening for constitutional X and Y chromosome aneuploidies.

SUMMARY AND EXPLANATION

In the management of many hematological malignancies, bone marrow transplantation (BMT) is a critical therapeutic strategy. Common cases treated with BMT include CML, AML, ALL, MDS, MPD, and occasionally chronic lymphocytic leukemia (CLL).[1,2] Although many individuals appear to form a stable chimeric state between donor and recipient bone marrow, others form an unstable one and the malignancy eventually recurs, with an increasing number of recipient cells appearing in the bone marrow or peripheral circulation.

Following the transplantation, an estimate of the proportions of donor and recipient cells can be used to assess the success of engraftment, to detect the presence of clonal neoplasms, and to diagnose recurrence. This is especially true for opposite-sex BMTs. Current methods used to assess engraftment success after BMT include standard cytogenetic analysis.[3]

Standard cytogenetic analysis involves karyotyping the bone marrow and identifying the donor and recipient cells by sex chromosome or chromosomal heteromorphic differences between donor and recipient. Metaphase spreads are prepared from unstimulated bone marrow after 24 to 48 hours in culture and stained with quinacrine dihydrochloride or Giemsa by conventional methods. Depending on the institution, standard cytogenetic analyses are typically performed on 20 to 30 mitotic cells. Recipients of opposite-sex BMT are studied by examination of the sex chromosome constitution in metaphase spreads (XX=female, XY=male). In bone marrow specimens from recipients of opposite-sex BMT, if one or more metaphase spreads is of donor origin, the specimen is positive for donor cells. The proportion of XX and XY cells can be used to assess the success of engraftment. A level of 100% donor cells is usually associated with complete engraftment. No clinical significance is emphasized when only 1 metaphase spread showing recipient cells is found.

An advantage of standard cytogenetic analysis is that if host cells are found, banding techniques can be used to detect chromosome abnormalities characteristic of the leukemic clone to document disease recurrence after bone marrow transplant. However, standard cytogenetic analysis is limited in detecting mixed chimerism in small proportions and requires examining a large number of metaphases to increase its sensitivity.[4] Analysis of 32 metaphases is required to exclude chimerism of 9% or greater with 95% confidence;[5] analysis of 459 or more metaphases is required to exclude chimerism of 1% with 95% confidence.[5] From a practical standpoint it is difficult to exclude levels of chimerism less than 10% using standard cytogenetic methods.

Furthermore, karyotype analysis requires actively dividing cells, and thus is restricted to proliferating cell populations. A lack of dividing cells due to marrow hypocellularity is the primary cause of failure in standard cytogenetic analysis.

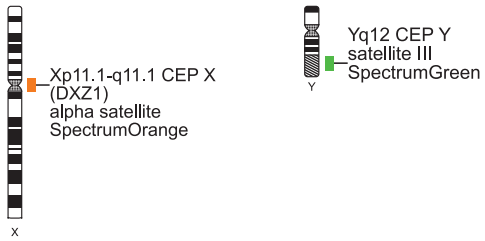
Use of FISH in conjunction with standard cytogenetics may provide a higher level of analytic sensitivity because of the ease of evaluating a large number of cells; generally it is possible to assess at least 500 interphase nuclei in any given specimen. Because of the large number of cells analyzed and the reported increase in analytic sensitivity of the FISH assay, FISH analysis should provide a greater precision of the estimated ratio of donor: recipient cells than standard cytogenetic analysis. However, this remains highly dependent on the integrity of the test reagents used, optimal analytic performance of the assay by the laboratory, and the recognition of suboptimal results.

PRINCIPLES OF THE PROCEDURE

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

Tissue consisting of interphase nuclei or metaphase spreads is attached to glass slides using standard cytogenetic protocols. The resulting specimen DNA is denatured to its single stranded form and then allowed to hybridize with the CEP X/Y DNA probe. Following hybridization, the excess and unbound probe is removed by a series of washes and the chromosomes and nuclei are counterstained with the DNA specific stain DAPI (4,6 diamidino-2-phenylindole) that fluoresces blue. Hybridization of the CEP X/Y DNA probe is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters allowing visualization of the intense orange fluorescent signal concentrated at the centromere of chromosome X, the intense green fluorescent signal concentrated at the Yq12 region of chromosome Y and the blue counterstained chromosomes and nuclei. The enumeration of chromosomes X and Y is conducted by microscopic examination of interphase and/or metaphase nuclei. The fluorescently stained centromeres of chromosome X and satellite III DNA of chromosome Y stand out brightly against the general blue fluorescence of the nuclear DNA provided by the DAPI counterstain. Relative to standard cytogenetic methods, the CEP procedure provides a higher percentage of interpretable nuclei per slide and enables visual enumeration of chromosomes X and Y within the nuclei.

The assay results are reported as the percentage of nuclei with an XX, XY and other signals among all cells with at least one fluorescent signal. Each orange fluorescent signal corresponds to the centromere of a chromosome X; each green fluorescent signal corresponds to the satellite III DNA of chromosome Y.



The CEP X DNA probe (DXZ1 locus) is a SpectrumOrange directly labeled fluorescent DNA probe specific for the AT rich alpha satellite DNA sequence at the centromeric region of chromosome X (Xp11.1-Xq11.1). The CEP Y DNA probe (DYZ1 locus) is a SpectrumGreen directly labeled fluorescent DNA probe specific for the satellite III DNA at the Yq12 region of chromosome Y. This assay is designed for the detection and quantification of chromosomes X and Y in both interphase nuclei and metaphase spreads by FISH.

REAGENTS AND INSTRUMENTS

Materials Provided

This kit contains 4 reagents in quantities sufficient to process approximately 20 assays. An assay is defined as one 22 mm × 22 mm target area.

Table 1. CEP X SpectrumOrange/Y SpectrumGreen DNA Probe Kit (Part No. 30-161050, List No. 07J20-050)

Component	Composition	Part No.	Contents	Storage
CEP X/Y DNA Probe: E. coli plasmid (The probe is pre-denatured)	14 ng/µL SpectrumOrange fluorophore labeled alpha satellite DNA X probe and SpectrumGreen labeled satellite III DNA Y probe pre-mixed with Blocking DNA and Hybridization Buffer. (dextran sulfate, formamide, SSC)	30-171050	220 µL/ 1 vial	–20°C protect from light
DAPI II Counterstain	125 ng/mL DAPI (4,6-diamidino-2-phenylindole) in phenylenediamine dihydrochloride, glycerol, and buffer.	30-804841	300 µL/ 1 vial	–20°C protect from light
NP-40	non ionic detergent	30-804818	1 mL/1 vial	–25 to 30°C
20X SSC	sodium chloride and sodium citrate	30-805850	66 g/ 1 container	–20°C to 25°C

Table 2. CEP X SpectrumOrange/Y SpectrumGreen DNA Probe Kit with ProbeChek Control Slides (Part No. 32-161050, List No. 07J22-050)

Kit contents include the following ProbeChek slides in addition to the components cited in **Table 1**.

Component	Composition	Part No./ List No.	Contents	Storage
ProbeChek Control Slides Low Level Male 5% XY/ 95% XX	Fixed cultured human lymphoblast male and female cells mixed in appropriate ratio for approximately 5% male cells and 95% female cells applied to glass microscope slides.	30-805012 /07J21-012	5 slides (10 target areas)	–20°C, desiccated
ProbeChek Control Slides Low Level Female 95% XY/ 5% XX	Fixed cultured human lymphoblast male and female cells mixed in appropriate ratio for approximately 5% female cells and 95% male cells applied to glass microscope slides.	30-805011 /07J21-011	5 slides (10 target areas)	–20°C, desiccated

STORAGE AND HANDLING

Store the unopened CEP X/Y DNA probe kit as a unit at –20°C protected from light and humidity. The 20X SSC salts and NP-40 may be stored separately at room temperature. Store the ProbeChek Control Slides at –20°C in a sealed container with desiccant to protect them from humidity. Expiration dates for each of the unopened kit components are indicated on the individual component labels. These storage conditions apply to both opened and unopened components.

Materials Required But Not Provided

NOTE: Where storage conditions are not listed in this insert or the product label store reagent per vendor recommendations.

Laboratory Reagents

- Ultra-pure grade formamide.
- Ethanol (100%). Store at room temperature.
- Concentrated (12N) HCl
- 1N NaOH
- Purified water (distilled or deionized or Milli-Q). Store at room temperature.
- Fixative (3:1 methanol:acetic acid). Prepare fresh daily.
- Drierite and Nitrogen gas

Laboratory Equipment

- Fluorescence microscope equipped with recommended filters
- Phase contrast light microscope
- Precleaned microscope slides
- Slide warmer (45 to 50˚C)
- 22 mm × 22 mm glass coverslips
- Microliter pipettor (1 to 10 µL) and sterile tips
- Polypropylene microcentrifuge tubes (0.5 mL or 1.5 mL)
- Timer
- Magnetic stirrer
- Vortex mixer
- Microcentrifuge
- Graduated cylinder
- Water baths (67 ± 2˚C and 73 ± 1˚C)
- Air incubator (42˚C)
- Diamond-tipped scribe
- Humidified chamber
- Forceps
- Disposable syringe (5 mL)
- Coplin jars (6) Suggested type: Wheaton Product. No. 900620 vertical staining jar
- pH meter and pH paper
- Calibrated thermometer
- Wire test tube racks
- Rubber cement
- 0.45 µm pore filtration unit

Microscope Equipment and Filters

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 "tune-ups" by the manufacturer's technical representative, especially alignment of the lamp, if required, are advisable.

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, if required.

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 25X or 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 40X, 63X or 100X oil immersion achromat type objective.

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

Filters: Multi-bandpass fluorescence microscope filter sets optimized for use with the CEP DNA probe kits are available from Abbott Molecular Inc. for most microscope models. A triple bandpass DAPI/Green/Orange filter set is recommended for the CEP X/Y kit. This filter configuration allows the simultaneous excitation and emission of the SpectrumOrange, SpectrumGreen and DAPI fluorophores. The CEP X/Y probe hybridization to its 2 target chromosomes is marked by orange and green fluorescence. All of the other DNA will fluoresce blue with the DAPI stain.

Preparation of Working Reagent Solutions

20X SSC

To prepare, add together:

66 g	20X SSC
200 mL	Purified water
250 mL	Final volume

Mix thoroughly. Measure pH at room temperature with a pH meter. Adjust to pH 5.3 with concentrated HCl, if necessary. Bring the total volume to 250 mL. Filter through a 0.45 µm filtration unit. Store in a covered container at room temperature for up to 6 months.

Denaturing Solution

To prepare, add together:

49 mL	Formamide
7 mL	20X SSC pH 5.3
14 mL	Purified water
70 mL	Final Volume

Mix well and place in a glass Coplin jar. Measure pH at room temperature with a pH meter. Verify pH is between 7.0 to 8.0. Store in a covered container at 2 to 8˚C. This solution can be used for up to 1 week. Check pH prior to each use.

Ethanol Wash Solutions

Prepare v/v dilutions of 70%, 85%, and 100% using 100% ethanol and purified water. Store at room temperature in tightly capped containers. Dilutions may be used for one week unless evaporation occurs or the solution becomes diluted due to excessive use.

0.4X SSC Wash Solution

To prepare, add together:

950 mL	Purified water
20 mL	20X SSC pH 5.3
1000 mL	Final Volume

Mix thoroughly. Measure pH at room temperature with a pH meter. Adjust to pH 7.0 to 7.5 with 1N NaOH, if necessary. Adjust volume to 1 liter with water. Filter through 0.45 µm pore filtration unit. Store unused solution in a covered container at room temperature for up to 6 months. Discard solution that was used in the assay at the end of each day.

0.1% NP-40 in 2X SSC

To prepare, add together:

100 mL	20X SSC pH 5.3
849 mL	Purified water
1 mL	NP-40
1000 mL	Final Volume

Mix thoroughly. Measure pH at room temperature with a pH meter. Adjust the pH to 7.0 to 7.5 with 1N NaOH. Adjust volume to 1 liter with water. Filter through 0.45 µm pore filtration unit. Add 70 mL to a Coplin jar and maintain at room temperature. Store unused solution in a covered container at room temperature for up to 6 months. Discard solution that was used in the assay at the end of each day.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

1. For In Vitro Diagnostic Use
2. The ProbeChek Control Slides to be used with this kit are manufactured from human cultured lymphoblast cells that have been fixed multiple times in a solution of methanol:acetic acid (3:1). *Because it is often impossible to know which might be infectious, all human specimens and control slides should be treated with universal precautions. Guidelines for specimen handling are available from the US Centers for Disease Control and Prevention.*[6]
3. Hybridization conditions may be adversely affected by the use of reagents other than those provided or recommended by Abbott Molecular.
4. Failure to follow all procedures for slide denaturation, hybridization and signal enumeration may cause unacceptable or erroneous results.
5. Fluorophores are readily photobleached by exposure to light. To limit this degradation, handle all solutions 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 subdued lighting to avoid direct light projecting onto the fluorophore.
6. CEP X/Y DNA Probe contains formamide, a teratogen. Avoid contact with skin and mucous membranes. Refer to MSDS for more information.
7. The use of a calibrated thermometer is strongly recommended for measuring temperatures of solutions, waterbaths, and incubators as these temperatures are critical for optimum product performance.
8. All hazardous materials should be disposed of according to your institution's guidelines for hazardous disposal.

CEP X SpectrumOrange/Y SpectrumGreen DNA Probe

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,[11] OSHA Standards on Bloodborne Pathogens,[12] CLSI Document M29-A3,[13] and other appropriate biosafety practices.[14] 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.[11]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[14]

Danger

Hazard-determining components of labeling: Formamide

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.
P281	Use personal protective equipment as required.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.
P501	This material and its container must be disposed of in a safe way.

NP-40

Danger

Hazard-determining components of labeling: Polyethylene glycol octylphenyl ether

H302	Harmful if swallowed.
H318	Causes serious eye damage.
H412	Harmful 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.
P501	This material and its container must be disposed of in a safe way.

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

Material Safety Data Sheets (MSDS) for all reagents provided in the kits are available upon request from the Abbott Molecular Technical Services Department (1-800-553-7042 in the US and from outside of the US +49-6122-580).

SPECIMEN COLLECTION, PROCESSING, STORAGE AND SLIDE PREPARATION

Specimen Collection and Processing

Bone marrow collection should be performed according to the laboratory's institution guidelines. The AGT Cytogenetics Laboratory Manual contains recommendations for specimen collection and culturing.[7] The AGT recommends collection of bone marrow in either sodium-heparinized transport medium or a sodium-heparinized vacutainer.[7] According to the AGT Manual, both containers are acceptable. Bone marrow specimens should be free of significant blood contamination, since the presence could dilute the bone marrow and alter the assay results with respect to the donor:recipient ratio.

The AGT Manual recommends that the specimens be transported to the cytogenetics laboratory and cultures of the bone marrow should be initiated immediately. The AGT Manual contains a number of recommendations for specimen culturing and harvesting. Bone marrow should not be frozen prior to culturing or harvesting. Harvested bone marrow can be immediately used to prepare slides, or stored in fixative at −20°C.[7]

Icteric or 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.

Slide Preparation from Fixed Cell Pellets

The following method may be used for preparing slides from cultured specimens.

1. Place a water bath and humidifier within a humidity containment device that is equipped with a front access. Cover the front of the containment device loosely with plastic wrap, but do not completely block access to the interior. If the room hygrometer reading is below 45%, a humidifier should be used.
2. Prewarm the water bath to 67±2°C. Place test tube racks in the center of the water bath so that they do not touch the sides of the bath. Maintain the water level to the top of the test tube rack throughout the procedure.
3. Prepare the cell pellet with fixative so that the suspension is slightly cloudy.
4. Clean a microscope slide by flooding both sides of the slide with 70% ethanol (use a squeeze bottle). Wipe the slide dry by drawing a laboratory wipe down the length of the slide from the labeled end. Use a pencil to label a slide with a painted blaze.
5. Dip the cleaned slide in a Coplin jar containing fixative. Tilt the slide to evenly coat its upper surface with fixative.
6. Immediately hold the slide over the water bath. Holding a Pasteur pipette 2 to 4 inches above the slide, expel 3 to 4 drops of cell specimen suspension along the length of the slide.
7. Place the slide, specimen side up, on the top of the test tube rack in the water bath. Let the slide dry for 10 minutes.
8. Remove the slide from the test tube rack and view under a phase contrast microscope. Examine the number of interphases per field, under low power (10X objective). A minimum of 100 cells per low power field is required for optimum assay results. Adjust the cell specimen suspension with fresh fixative to achieve the recommended number of interphase nuclei.
9. Gently outline the area containing the interphase nuclei on the back of the slide with a diamond-tipped scribe. Since a coverslip (22 mm × 22 mm) is to be used to form the hybridization zone, the area outlined should be within that of the coverslip. Up to 2 coverslips may be applied per slide.
10. Place the specimen slides in a slide box.
11. Age the specimen slides at room temperature for 24 hours, with the slide box uncovered, before hybridization or storage.

Slide Storage

Place the prepared slides in a covered slide box. Seal the slide box in a plastic bag purged with nitrogen gas and containing approximately 1 tablespoon of Drierite. Store at −20°C prior to hybridization.

ASSAY PROCEDURE: FISH PROCEDURE SUMMARY

Denaturation of Specimen DNA:

1. Prewarm the hybridization chamber (an airtight container) to 42°C by placing it in the 42°C incubator prior to slide preparation.
2. Add denaturing solution to Coplin jar and place in a 73±1°C water bath for at least 30 minutes. Verify the solution temperature before use.
3. Denature the specimen DNA by immersing the prepared slides in the denaturing solution at 73±1°C for 5 minutes. Do not denature more than 4 slides at one time per Coplin jar.
4. Using forceps, remove the slide(s) from the denaturing solution and immediately place into a 70% ethanol wash solution at room temperature. Agitate the slide to remove the formamide. Allow the slide(s) to stand in the ethanol wash for 1 minute.
5. Remove the slide(s) from 70% ethanol. Repeat step 4 with 85% ethanol, followed by 100% ethanol.
6. Drain the excess ethanol from the slide by touching the bottom edge of the slide to a blotter and wipe the underside of the slide dry with a laboratory wipe.

7. Place the slide(s) on a 45 to 50°C slide warmer no more than 2 minutes before you are ready to apply the probe solution.

NOTE: If the timing of the hybridization is such that the slide is ready more than 2 minutes before the probe is ready, the slide should remain in the jar of 100% ethanol. Do not air dry a slide before placing it on the slide warmer.

Probe Preparation

1. Allow the probe to warm to room temperature, thus decreasing the viscosity and allowing for accurate pipetting.
2. Vortex to mix. Spin the tubes briefly (1 to 3 seconds) in microcentrifuge to bring the contents to the bottom of the tube. Gently vortex again to mix.

NOTE: The probe is pre-denatured and is ready to apply to the denatured target area on the specimen slide.

Hybridization

1. Apply the 10 µL aliquot of probe solution to the target area of the slide. Immediately, place a 22 mm × 22 mm glass coverslip over the probe solution and allow the solution to spread evenly under the coverslip. Air bubbles will interfere with hybridization and should be avoided.

NOTE: Do not pipet probe solution onto multiple target areas before applying the coverslips.

2. Place the slide into the prewarmed 42°C hybridization chamber and cover the chamber with a tight lid.
3. Place the chamber containing the slide into the 42°C incubator and allow hybridization to proceed for at least 30 minutes.

NOTE: Longer hybridization time may be required for sufficient signal intensity in some specimens. Incubations may be performed overnight (up to 16 hours). For incubations longer than 1 hour, the coverslip must be sealed using a removable sealant such as rubber cement and the hybridization chamber must be humidified. The procedure is described below.

- Draw rubber cement into a 5 mL syringe. Exude a small amount of rubber cement around the periphery of the coverslip overlapping the coverslip and the slide, thereby forming a seal around the coverslip.
- Place the slide into a humidified hybridization chamber (an air tight container with a piece of damp blotting paper or paper towel approximately 1 in. × 3 in. taped to the side of the container).
- Cover the chamber with a tight lid and incubate 1 to 16 hours, as desired.
- Following incubation, remove the rubber cement from the coverslip by pulling up on the rubber cement.

Post-hybridization Washes

1. Add 0.4X SSC (pH 7.0 to 7.5) to a Coplin jar. Prewarm the 0.4X SSC solution by placing the Coplin jar in the 73±1°C water bath for at least 30 minutes or until the solution temperature has reached 73±1°C.

NOTE: If more than 4 slides have been hybridized they must be washed in more than 1 batch. The temperature of the wash solution must return to 73±1°C before washing each batch.

2. Remove the coverslip from the target area of the first slide and immediately place the slide into the Coplin jar containing 0.4X SSC, 73±1°C. Agitate the slide for 1 to 3 seconds. Repeat for the other 3 slides and incubate for 2 minutes at 73±1°C.

NOTE: Do not remove the coverslips from several slides before placing any of the slides in the wash bath. Begin timing the 2 minute incubation when the last slide has been added to the wash bath.

3. Remove each slide from the wash bath and place in the jar of 2X SSC/0.1% NP-40 at room temperature for 5 to 60 seconds, agitating for 1 to 3 seconds as the slides are placed in the bath.
4. Allow the slide to air dry in the dark. (A closed drawer or a shelf inside a closed cabinet is sufficient.)
5. Apply 10 µL of DAPI II counterstain to the target area of the slide and apply a glass coverslip. Store the slide(s) in the dark prior to signal enumeration.

Storage

Store hybridized slides (with coverslips) at −20°C in the dark. Under these conditions the slides can be stored for up to 12 months without significant loss in fluorescence signal intensity. For long term storage, the coverslips may be sealed to prevent desiccation and the slides stored at −20°C.

Signal Enumeration

Assessing Slide Adequacy

Evaluate slide adequacy using the following criteria:

- Probe Signal Intensity: The signal should be bright, distinct, and easily evaluable. Signals should be in either bright, compact, oval shapes or stringy, diffuse, oval shapes.
- Background: The background should appear dark or black and free of fluorescence particles or haziness.
- Cross-hybridization/Target Specificity: The probe should hybridize and illuminate only the target (centromere of chromosome X or Yq12 region of chromosome Y). Metaphase spreads should be evaluated to identify any cross-hybridization to non-target sequences. At least 98% of cells should show 1 or more signals for acceptable hybridization (see guidelines for signal enumeration below).

If any of the above features are unsatisfactory, consult **Table 3. Troubleshooting Guide**, and process a fresh slide.

Selection of optimum viewing area and evaluable nuclei

Use a 25X objective to scan the hybridized area and examine the specimen distribution. Select an area where the specimen is distributed sparsely, few interphase nuclei or metaphase spreads are overlapping, and several interphase nuclei or metaphase spreads can be scanned within a viewing field. Avoid areas where the distribution of cells is dense, cells are overlapped, or the nuclear border of individual nuclei is unidentifiable. Avoid areas which contain clumps of cells. Enumerate only those cells with discrete signals.

Enumeration scan

Using a 40X or 63X objective, begin analysis in the upper left quadrant of the selected area and, scanning from left to right, count the number of signals in each evaluable metaphase spread or within the nuclear boundary of each evaluable interphase cell. Areas on the slide with a high cell density should be randomly skipped in order to scan the entire target area. Continue the scanning until 500 interphase nuclei are enumerated and a minimum of 20 metaphase spreads are counted and analyzed. If greater than 5% of the nuclei show no hybridization signal after enumerating 200 nuclei, the slide should be designated as a hybridization failure and no results should be reported.

Interphase Enumeration

Enumerate the fluorescent signals in each evaluable interphase nucleus using a 40X or 63X objective. Follow the signal counting guidelines in **Figure 1**. Objectives with higher magnification (eg, 63X or 100X) should be used to verify or resolve questions about split or diffused signals.

- Two signals that are in close proximity and approximately the same sizes but not connected by a visible link are counted as 2 signals.
- Count a diffuse signal as one signal if diffusion of the signal is contiguous and within an acceptable boundary.
- Two small signals connected by a visible link are counted as 1 signal.
- Enumerate the number of nuclei with 0, 1, 2, 3, 4, or >4 signals (for both X and Y signals) and record the counts in a 2-way table. Count nuclei with 1 or more FISH signals of either color. If the accuracy of enumeration is in doubt, repeat the enumeration in another area of the slide.
- Do not enumerate nuclei with uncertain signals.

Metaphase Enumeration

- The metaphase spread should have chromosomes that are well separated from each other but are clearly from the same cell. Select a minimum of 20 good quality, complete metaphase spreads with well defined, non-overlapping chromosomes for chromosome enumeration and analysis.
- The CEP X/Y DNA Probe signal will be visible as a distinct fluorescent signal located near the centromere region of the X chromosome and the Yq12 region of chromosome Y. The CEP signal may appear split (2 smaller signals in close proximity) if the chromatids are separated. Chromatid separation occurs when the cell is in the later stages of mitosis (between metaphase and anaphase). The split signal found on each of the 2 chromatids should be counted as one signal. Follow general magnification and scanning guidelines as indicated above in the "Interphase Enumeration".
- In addition to enumerating the X/Y signals, metaphase cells should be assessed to verify locus specificity of the probes and to assure that there are no cross-hybridizing sequences at alternate chromosomal locations.

Table 3. Troubleshooting Guide

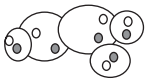
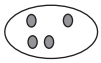
Problem	Probable Cause	Solution
• No signal or weak signals	• Inappropriate filter set used to view slides	• Use correct filters
	• Microscope not functioning properly	• Call microscope manufacturer's technical representative
	• Improper lamps (ie, Xenon or Tungsten)	• Use a mercury lamp (100-watt recommended)
	• Mercury lamp too old	• Replace with a new lamp
	• Mercury lamp misaligned	• Realign lamp
	• Dirty and/or cracked collector lenses	• Clean or replace lens
	• Dirty or broken mirror in lamp house	• Clean or replace mirror
	• Inappropriate objectives	• Use recommended objectives
	• Probes not denatured properly	• Check temp. of 73±1°C water bath
	• Hybridization conditions inappropriate	• Check temp. of 42°C incubator
		• Increase hybridization time to 1 hour
	• Wash conditions inappropriate	• Check temp. of 73±1°C water bath
		• Check formulation of wash baths (eg, pH)
	• Air bubbles trapped under coverslip and prevented probe access	• Apply coverslip by first touching the surface of the hybridization mixture
	• Probes improperly stored	• Store probes at –20°C in darkness
• Low signal specificity	• Hybridization conditions inappropriate	• Check temperature of 42°C incubator
	• Wash temperature too low	• Maintain wash temperature at 73±1°C
• High slide background	• Metaphase spreads were aged by baking or contain a lot of cytoplasm	• Increase slide denaturation time to 10 minutes
	• Cellular debris in cell preparation	• Wash cell preparation 5 times with fresh fixative and repeat Slide Preparation procedure
	• Specimen DNA not "clean"	• Replace 0.4X SSC post hybridization wash with formamide wash as follows 1. wash slide(s) 3X for 10 minutes each in 50% formamide/2X SSC pH 7.5 to 8.0 at 46±1°C 2. wash slide(s) 1X for 10 minutes in 2X SSC at 46±1°C 3. wash slide(s) 1X for 5 minutes in 2X SSC/0.1% NP-40 at 46±1°C
	• Use of long pass filters which pass a lot of light	• Switch to filters with smaller bandwidths or a multi-bandpass filter
	• Washes at wrong temperature or wrong formulation	• Check bath temperature, pH, and/or formulation
	• Slides not properly cleaned prior to dropping target cells onto them	• Dip slides in ethanol and wipe with laboratory wipe prior to dropping cells

Table 3. Troubleshooting Guide (Continued)

Problem	Probable Cause	Solution
• "Distorted" chromosome morphology	• Slides dried too quickly during sample preparation	• Increase relative humidity during sample slide preparation
		• Increase temperature of water bath during sample slide preparation
	• Slides too fresh prior to denaturation	• Increase sample slide drying time
		• Age slides at least 24 hours at room temperature prior to denaturation
	• Slides not thoroughly dry prior to denaturation	• Warm slides at 45°C for 10 to 15 minutes prior to denaturation
	• Temperature too high in denaturing bath	• Check water bath temperature
• Excessively bright signal	• Probe concentration too high for your microscope	• Try to block some of the signal by placing a neutral density filter in the excitation pathway

Figure 1. Image Examples and Signal Counting Guidelines

Key: ○ = green probe
● = orange probe

1		Do not count. The nuclei are overlapping and all areas of both nuclei are not visible.
2		Count as 1 orange signal and 1 green signal. The orange signal is diffuse.
3		Do not count. The nuclei are too close together to determine cell boundaries.
4		Count as 1 orange signal and 1 green signal. The orange signal is split.
5		Count as 1 orange signal and 2 green signals. One green signal is split and the orange signal is split.
6		Count as 2 orange signals and 1 green signal.
7		Count as 3 orange signals and 1 green signal.
8		Count as 4 orange signals.

Quality Control

Use of Control Slides

Low level male (5% XY/95% XX) and low level female (5% XX/95% XY) control slides must be run concurrently with patient slides to monitor assay performance and to assess the accuracy of signal enumeration. Controls should be run on each day of FISH testing and with each new probe kit lot. The ProbeChek Control Slides are recommended.

Slide adequacy and signal enumeration should be assessed using the criteria described above in the signal enumeration section. The criteria for slide adequacy must be satisfied and the signal enumeration results should be within the specifications on the data sheets provided with the control slides for acceptable test performance.

If control slides fail to meet the slide acceptance criteria, the assay may not have been performed properly or the CEP X SpectrumOrange/CEP Y SpectrumGreen DNA Probe Kit component(s) may have performed inadequately. A repeat analysis with fresh control slides and patient specimen slide(s) may be necessary. Consult the **Troubleshooting Guide** in **Table 3** for probable causes and the actions needed to correct the problems.

If control slides meet the acceptance criteria but the enumeration values are outside the specified range, the enumeration may not have been performed correctly and an independent, repeat assessment of the same slide may be appropriate.

In no case should routine FISH test results be reported if assay controls fail. For clinical specimens, when interpretation of the hybridization signal is difficult and there is insufficient specimen sample for re-assay, the test is uninformative. If there are insufficient cells for analysis, the test is uninformative.

NOTE: The ProbeChek packages contain 5 low level male slides (Part No. 30-805012, List No. 07J21-012) or 5 low level female slides (Part No. 30-805011, List No. 07J21-011). The acceptable range for the % XY/XX nuclei is indicated on the specification data sheet that accompanies these slides.

TEST INTERPRETATION

Results on enumeration of 500 interphase nuclei are reported as the number and percentage of interphase nuclei with XX, XY and "other" signals. "Other" signals include XXY, XYY, XXX, XXXX, XXYY, X, and Y; the % of cells with XX and XY signals are the primary variables of interest. Unless standard cytogenetics and FISH assay with CEP X/Y in pre-BMT specimens indicate the presence of "other" signals from either donor or recipient, the sum of the percentages of cells with XX and XY should be greater than 95%. Bone marrow specimens from recipients of opposite-sex BMT with >0.6% donor cells are considered to be positive for the presence of donor cells. Bone marrow specimens from recipients of opposite-sex BMT with ≤0.6% are considered negative, although the presence of donor cells can not be completely excluded.

Results on enumeration of 20 to 30 metaphases are reported as the number and percentage of metaphase spreads with XX and XY signals. Interpretation of the results follows the same rules as the standard cytogenetic analysis. Under these rules, a specimen is negative for the presence of donor cells if no metaphases of donor origin are found in 20 to 30 metaphases. If one or more metaphase of donor origin is found, the specimen is positive for donor cells.

The proportion of XX and XY cells can be used to assess the success of engraftment in opposite-sex bone marrow transplants. For metaphase analysis, no clinical significance is associated with the detection of only one recipient cell. Although FISH interphase analysis may be able to detect as few as 1% recipient (or donor) cells, the clinical significance of 1% vs. 5% recipient cells is not known. Although significant change in the proportion of recipient:donor cells may be of clinical significance, this device has not been validated for monitoring engraftment success.

If standard cytogenetic and FISH results do not agree or the results are not consistent with the clinical findings, the basis for any discrepancies should be carefully examined. Discrepancies in results among test methods may be due to inaccurate results from one or more of the test methods, differences in analytic sensitivity/specificity among the methods, actual differences in chromosome X/Y status among the cell populations assessed with the different methods (eg, cycling metaphase cells vs. non-cycling interphase cells), among others. When the percentage of XX or XY interphase nuclei are near the cutoff point (< 5%),[15,16] the results should be interpreted with caution and further assessment of the test specimen may be required. Repeat FISH (run concurrently with QC material) and/or repeat standard cytogenetic analysis with the remaining sample may be useful to assess the possibility of incorrect test results. If the basis for any discrepancies in the test results is not determined or if the test results are not consistent with the clinical findings, a consultation between the cytogeneticist and the treating physician is warranted.

LIMITATIONS

1. The CEP X/Y DNA Probe Kit has been optimized only for identifying chromosomes in interphase nuclei or metaphase spreads from bone marrow specimens.
2. This assay identifies only the proportion of donor and recipient cells in bone marrow specimens from recipients of opposite-sex bone marrow transplantation. It does not distinguish between malignant and normal cells; it is not designed to detect structural or other chromosome abnormalities in malignant clones, which is possible with standard cytogenetics.
3. The Y chromosome is sometimes lost in bone marrow cells of elderly males regardless of whether the specimen is from a donor, a recipient, or collected from a patient in the post-bone marrow transplantation period.[8]
4. It is important to have pretransplant cytogenetic results on both donor and recipient for the following reasons: (1) There are rare male patients who may have an unusual Y chromosome (lacking the Yq heterochromatic region) which cannot be identified with the CEP X/Y assay. (2) Some individuals may have target sequences at alternate chromosome locations that hybridize to the CEP X or Y probes. This has not been investigated for CEP X, however, chromosome polymorphisms which hybridize with the Y probe occur with a frequency of 1 in 2,000.[9] Such cases may be detected by CEP X/Y metaphase analysis and sometimes by standard cytogenetic analysis. (3) Constitutional sex chromosome aneuploidy, including mosaicism, present in either donor or recipient can complicate signal enumeration and test interpretation.
5. In a male donor or recipient with a 46, XY, -Y, +X karyotype, a certain percentage of cells with XX signals will be detected by CEP X/Y.
6. If significant peripheral blood contamination is present in the bone marrow specimen, the blood may dilute the specimen. It is important to recognize the potential effects this dilution effect may have on the FISH assay results; dilution of the bone marrow with blood may alter the donor:recipient cell ratio.
7. The CEP X/Y assay has been validated only for use with unstimulated, cultured bone marrow specimens obtained from recipients of opposite-sex BMT. It is not intended for chromosome X and Y enumeration in other patient populations or with other test matrices such as amniocytes, chorionic villi, fibroblasts, tumor cells, long term cultures, among others.
8. FISH assay results may not be informative if the specimen quality and/or specimen slide preparation is inadequate.
9. This device is not intended for use in subjects with like-sex bone marrow transplants or for use in diagnostic testing or screening for constitutional X and Y chromosome aneuploidies.
10. Residual fetal cells may potentially exist in either donor or recipient cells, however the levels at which these cells exist is likely to be below the levels of detection of both standard cytogenetics and FISH.
11. The CEP X/Y assay has not been validated for monitoring engraftment status.
12. The clinical significance and interpretation of FISH results should be made in conjunction with proper controls, standard cytogenetic analysis, and within the context of the patient's medical history and other clinical findings.

EXPECTED VALUES

FISH interphase signal enumeration was performed on bone marrow specimens obtained from normal subjects and from subjects who had received opposite-sex bone marrow transplants to assess the expected percentage of cells with XY and XX signals for these two states and to determine the cutoff for defining the presence or absence of XX cells in males and XY cells in females. Each specimen was enumerated for the percentage of cells with XX, XY and "other" (eg, X0, XXY, XXYY, etc.) signals per cell.

Values Among Normal Specimens

FISH interphase analysis was performed on bone marrow specimens from 57 normal female and 71 normal male subjects. The signal distribution for this study population is summarized in **Tables 4** and **5**.

Table 4. Distribution of Percentage of Cells with X and Y Signals in 71 Normal Male Bone Marrow Specimens

	Percentage of Cells With								
	XX	**XY**	**X0**	**XXX**	**0Y**	**XXY**	**XYY**	**XXYY**	**XXXX**
Mean	0.04	98.51	1.03	0.00	0.21	0.10	0.02	0.06	0.00
SD	0.10	1.86	1.38	0.00	0.40	0.17	0.06	0.12	0.00

Table 5. Distribution of Percentage of Cells with X and Y Signals in 57 Normal Female Bone Marrow Specimens

	Percentage of Cells With								
	XX	**XY**	**X0**	**XXX**	**0Y**	**XXY**	**XYY**	**XXYY**	**XXXX**
Mean	97.38	0.01	2.33	0.14	0.004	0.00	0.00	0.00	0.07
SD	1.81	0.05	1.66	0.20	0.026	0.00	0.00	0.00	0.17

In cytogenetically normal bone marrow specimens, the percentage of cells with XY nuclei in males and XX nuclei in females, and the percentage of cells with XX nuclei in males and XY nuclei in females are 2 critical categories for assessing expected values. The average (±S.D.) percentages of cells with XY and XX nuclei in normal males were 98.51% (±1.86%) and 0.04% (±0.10%), respectively. The average (±S.D.) percentages of cells with XX and XY nuclei in normal females were 97.38% (±1.81%) and 0.01% (±0.05%), respectively. Thus, when the recommended enumeration guide is followed and practiced, the percent of cells with XY nuclei in normal male should be between 94.9% and 100% (95% CI) and XX nuclei in normal female bone marrow should be between 93.8% and 100% (95% CI); the percent of cells with XX nuclei in normal male should be between 0% and 0.24% (95% CI) and XY nuclei in normal female bone marrow should be between 0% and 0.20% (95% CI).

Values Among Recipients of Opposite-Sex Bone Marrow Transplant

A study was performed to assess the distribution of interphase FISH signals in 143 specimens from opposite-sex BMT recipients. The distribution of signals for these 143 subjects (71 female and 72 male recipients) is summarized in **Tables 6** and **7**.

Table 6. Distribution of Percentage of Cells with X and Y Signals in Bone Marrow Specimens from 71 Female Recipients of Opposite-Sex BMT

	Percent of Cells With								
	XX	XY	X0	XXX	0Y	XXY	XYY	XXYY	XXXX
Mean	5.88	92.99	0.008	0.025	0.14	0.001	0.011	0.073	0.014
SD	19.3	19.35	0.010	0.112	0.29	0.002	0.046	0.214	0.098

Table 7. Distribution of Percentage of Cells with X and Y Signals in Bone Marrow Specimens from 72 Male Recipients of Opposite-Sex BMT

	Percent of Cells With								
	XX	XY	X0	XXX	0Y	XXY	XYY	XXYY	XXXX
Mean	92.72	5.64	0.015	0.067	0.011	0.0002	0.006	0.003	0.067
SD	16.91	16.8	0.013	0.157	0.094	0.0011	0.033	0.024	0.174

In bone marrow specimens from recipients of opposite-sex BMT, the critical category is the percentage of XX nuclei in males and XY nuclei in females.

Cutoff Point for Defining Presence of Donor Cells after Opposite-Sex BMT

The other critical category in normal bone marrow specimens is the percent of cells with XX signals in normal males and XY signals in normal females. These percentages were used for the determination of a cutoff point for defining the presence or absence of opposite-sex bone marrow. The percent of cells with XX signals in males and XY signals in females was calculated for each of the 128 (57 female and 71 male) subjects in the pivotal study. To determine the normal reference range, a (one-sided) 95% confidence interval using a binomial distribution for the proportion of interphase cells with XX or XY was calculated; the upper boundary was 0.6%. To determine the cutoff point, the following table (**Table 8**) was derived by the method for calculating Confidence Limits for Proportions.[10]

Table 8. Cutoff Point for Classifying Presence of Donor Cells based on Number of XX Cells in Normal Males and XY Cells in Normal Females

number of opposite-sex cells found	500 cells counted
0	0.6%
1	1.0%
2	1.2%
3	1.6%
4	1.8%
5	2.1%

Based on the data above, a cutoff point of 0.6% was established for determining the presence of donor cells in cases of opposite-sex BMT.

Before clinical use of the CEP X/Y kit, the laboratory should verify its cutoff by analyzing and enumerating a minimum of 10 male and 10 female bone marrow specimens according to the instructions in the enumeration section of the package insert. The percent XX nuclei in the normal male specimens and the percent of XY nuclei in the normal female specimens should all be below the cutoff point of 0.6%. If this cutoff point is not appropriate for the user's institution, the user may choose to redefine this cutoff point by following the statistical procedure described above. Note that a sample size of 20 (10 male and 10 female) will not suffice for defining a new cutoff.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity and Specificity

Hybridization Efficiency

In a pivotal study, the average percentage of cells with only one hybridization signal was 0.012% (SD = 0.15%) on 143 bone marrow specimens. Thus, < 2% cells with only one signal is a realistic standard of acceptance.

Analytical Sensitivity

The analytical sensitivity of the CEP X/Y probe was tested in the reproducibility study described below. In that study, the 0% XY specimen was estimated with a mean (±S.D.) of 0.00% (±0.00%) XY nuclei and the 1% XY specimen, 0.94% (±0.32%). The 0% XX specimen was estimated with a mean (±SD) of 0.00% (±0.00%) XX nuclei and the 1% XX specimen, 0.95% (±0.34%). There was little overlap between the 0% and 1% specimens; the lower 95% confidence limit for the 1% specimen was 0.31% and 0.28% for XY and XX, respectively. The limit of detection for CEP X/Y is estimated to be 1.0%.

Analytical Specificity

Locus specificity studies were performed with metaphase spreads according to standard Abbott Molecular Quality Control (QC) protocols. A total of 65 metaphase spreads were examined sequentially by G-banding to identify chromosomes X and Y, followed by FISH. No cross-hybridization to other chromosome loci was observed in any of the 65 cells examined; hybridization was limited to the centromere of chromosome X and the Yq12 region of chromosome Y.

Reproducibility

To assess the reproducibility of the CEP X/Y interphase analysis for the percentage of cells with XX and XY signals, bone marrow specimens with approximately 0%/100%, 1%/99%, 5%/95%, 95%/5% XY/XX, 99%/1% and 100%/0% XY/XX were prepared. Inter-site, inter-lot, inter-day, and inter-observer reproducibility were assessed in a pivotal study with 2 of these bone marrow specimen mixtures (approximately 99%/1% and 100%/0% XY/XX,) and 2 mixtures of hematologically derived human cells with approximately 0%/100% and 1%/99% XY/XX. The percentage of cells with XX and XY signals were evaluated according to the instructions for signal enumeration in the package insert. Using ANOVA, significant, site-to-site and observer-to-observer variations were observed, reflecting the subjectivity of the visual enumeration process. In addition to the pivotal study, 4 bone marrow specimens with approximately 0%/100%, 1%/99%, 5%/95% and 95%/5% XY/XX were prepared and analyzed at one site. The mean, standard deviation, and percent CV of the observed percentage of XX and XY nuclei for the pivotal study specimens, and these additional bone marrow specimens are shown in **Tables 9 through 13**.

Table 9. Precision of the Observed % XY/XX Signaled Nuclei Detection

Specimen Level of XY/XX:		N	Mean (%)		Standard Deviation (%)		Coefficient of Variation (%)	
			XY	XX	XY	XX	XY	XX
0%	100%	10	0.00	97.4	0.00	1.18	—	1.21
1%	99%	20	0.88	97.2	0.48	2.00	54.8	2.06
5%	95%	20	4.90	94.9	0.99	0.99	20.2	1.04
95%	5%	10	95.0	4.96	1.60	1.60	1.68	32.3
99%	1%	24	98.3	0.95	0.41	0.34	0.41	36.3
100%	0%	24	99.0	0.00	0.47	0.00	0.48	—

Table 10. Summary Statistics of % XY/XX Nuclei by Study Site

Level of			Site #1		Site #2		Site #3	
XX	XY	Statistics	XX	XY	XX	XY	XX	XY
100%	0%	Mean	97.40	0.00				
		SD	1.18	0.00				
		CV(%)	1.21	—				
		n = 10						
99%	1%	Mean	97.20	0.88				
		SD	2.00	0.48				
		CV(%)	2.06	54.8				
		n = 20						
95%	5%	Mean	94.9	4.90				
		SD	0.99	0.99				
		CV(%)	1.04	20.2				
		n = 20						
5%	95%	Mean	4.96	95.0				
		SD	1.60	1.60				
		CV(%)	32.3	1.68				
		n = 10						
1%	99%	Mean	0.90	97.80	0.88	98.90	0.65	99.23
		SD	0.36	0.84	0.21	0.21	0.30	0.29
		CV(%)	39.40	0.86	24.24	0.22	45.79	0.29
		n = 8						
0%	100%	Mean	0.00	98.93	0.00	99.75	0.00	99.73
		SD	0.00	0.48	0.00	0.28	0.00	0.18
		CV(%)	—	0.48	—	0.28	—	0.18
		n = 8						

SD (Standard Deviation), CV(%) (Coefficient of Variation).

Table 11. Summary Statistics of % XY/XX Nuclei by Probe-Lot

Level of			Lot #1		Lot #2		Lot #3		Lot #4	
XX	XY	Statistics	XX	XY	XX	XY	XX	XY	XX	XY
100%	0%	Mean	97.40	0.00						
		SD	1.18	0.00						
		CV(%)	1.21	—						
		n=10								
99%	1%	Mean	97.20	0.88						
		SD	2.00	0.48						
		CV(%)	2.06	54.8						
		n = 20								
95%	5%	Mean	94.9	4.90						
		SD	0.99	0.99						
		CV(%)	1.04	20.2						
		n = 20								
5%	95%	Mean	4.96	95.0						
		SD	1.60	1.60						
		CV(%)	32.3	1.68						
		n = 10								
1%	99%	Mean	0.83	98.70	0.87	98.60	0.60	98.87	0.93	98.40
		SD	0.20	0.69	0.21	0.77	0.36	0.80	0.37	1.06
		CV(%)	23.60	0.70	24.14	0.78	59.63	0.81	39.90	1.08
		n = 6								
0%	100%	Mean	0.00	99.50	0.00	99.43	0.00	99.43	0.00	99.50
		SD	0.00	0.52	0.00	0.57	0.00	0.63	0.00	
		CV(%)	—	0.52	—	0.58	—	0.63	—	0.44
		n = 6								

SD (Standard Deviation), CV(%) (Coefficient of Variation).

Table 12. Summary Statistics of %XY/XX Nuclei by Assay Day

Level of			Assay Day #1		Assay Day #2		Assay Day #3		Assay Day #4	
XX	XY	Statistics	XX	XY	XX	XY	XX	XY	XX	XY
100%	0%	Mean	97.40	0.00						
		SD	1.18	0.00						
		CV(%)	1.21	—						
		n = 10								
99%	1%	Mean	97.20	0.88						
		SD	2.00	0.48						
		CV(%)	2.06	54.8						
		n = 20								
95%	5%	Mean	94.9	4.90						
		SD	0.99	0.99						
		CV(%)	1.04	20.2						
		n = 20								
5%	95%	Mean	4.96	95.0						
		SD	1.60	1.60						
		CV(%)	32.3	1.68						
		n = 10								
1%	99%	Mean	0.83	98.70	0.93	98.40	0.77	98.63	0.70	98.83
		SD	0.20	0.69	0.37	1.06	0.15	0.77	0.43	0.80
		CV(%)	23.60	0.70	39.90	1.08	19.64	0.79	61.94	0.81
		n = 6								
0%	100%	Mean	0.00	99.50	0.00	99.43	0.00	99.40	0.00	99.53
		SD	0.00	0.56	0.00	0.63	0.00	0.51	0.00	0.45
		CV(%)	—	0.57	—	0.63	—	0.51	—	0.45
		n = 6								

SD (Standard Deviation), CV(%) (Coefficient of Variation).

Table 13. Summary Statistics of % XY/XX Nuclei by Observer

Level of			Observer #1		Observer #2	
XX	XY	Statistics	XX	XY	XX	XY
100%	0%	Mean	98.00	0.00	96.80	0.00
		SD	0.71	0.00	1.31	0.00
		CV(%)	0.73	—	1.35	—
		n = 5				
99%	1%	Mean	95.4	0.80	99.00	0.96
		SD	0.93	0.53	0.45	0.48
		CV(%)	0.97	66.1	0.45	50.0
		n = 10				
95%	5%	Mean	95.2	4.48	94.56	5.32
		SD	0.65	0.53	1.19	1.18
		CV(%)	0.68	11.8	1.26	22.2
		n = 10				
5%	95%	Mean	5.16	94.84	4.76	95.20
		SD	2.36	2.36	0.61	0.61
		CV(%)	45.73	2.49	12.8	0.64
		n = 5				
1%	99%	Mean	0.80	98.50	0.82	98.78
		SD	0.33	1.04	0.29	0.46
		CV(%)	41.29	1.06	35.35	0.47
		n = 12				
0%	100%	Mean	0.00	99.45	0.07	99.58
		SD	0.00	0.65	0.23	0.42
		CV(%)	—	0.65	—	—
		n = 12				

SD (Standard Deviation), CV(%) (Coefficient of Variation).

Methods Comparison; Clinical Specimens

A multi-center, blinded, controlled, comparative study was conducted to characterize the performance of the CEP X/Y DNA probe kit in identifying the proportion of XX and XY cells, relative to standard cytogenetic analysis, in recipients of opposite-sex bone marrow transplants (BMT). Archived bone marrow specimens, which were previously evaluated by standard cytogenetic analysis, were selected from a total of 143 patients (72 males and 71 females), who were the recipients of opposite-sex BMTs. Consecutive specimens were selected and evaluated at 3 sites; site 1 provided and analyzed 40 specimens; site 2, 52 specimens; and site 3, 51 specimens. These specimens were derived from patients with 1 of the following diagnoses.

1. Chronic myelogenous leukemia **(CML)**: 69 specimens
2. Acute myeloid leukemia **(AML)** or Acute nonlymphocytic leukemia **(ANLL)**: 30 specimens
3. Myelodysplastic syndrome **(MDS)**: 7 specimens
4. Acute lymphoid leukemia **(ALL)**: 21 specimens
5. Hematological disorder not otherwise specified, but in which cytogenetics are commonly requested **(HDNOS)**: 16 specimens

All sites used unstimulated, cultured specimens for both standard cytogenetic and FISH analyses. Each site followed its own in-house protocol for standard cytogenetic analysis; FISH analyses were performed according to the instructions in the CEP X/Y DNA probe kit package insert. The number of donor and recipient cells were enumerated by FISH in a minimum of 20 metaphase and 500 interphase cells.

As expected for specimens with presumed sex chromosome chimerism after opposite-sex BMT, donor cells were detected in each of the 143 specimens by standard cytogenetic analysis. Interphase FISH analysis designated 143/143 specimens as positive for the presence of donor cells (100% relative sensitivity). FISH metaphase analysis detected donor cells in 141/141 specimens (100% relative sensitivity). The distribution of donor cells is shown in **Table 14** by site and method of analysis.

NOTE: Two specimens had no metaphase spreads for FISH analysis, thus the total number was 141, instead of 143.

Table 14. Distribution of Donor Signals by Site for each Method

	Distribution of Donor Cells		
Method	**Site 1**	**Site 2**	**Site 3**
Standard Cytogenetics	2.5% to 100%	10.0% to 100%	30.0% to 100%
Metaphase FISH	3.2% to 100%	30.0% to 100%	25% to 100%
Interphase FISH	10.4% to 98.8%	29.4% to 100%	21.3% to 100%

In addition to assessing the performance of FISH in the target population of patients with opposite-sex BMT, the ability of interphase and metaphase FISH to correctly designate specimens with like-sex BMT as negative was assessed in 153 patients with like-sex BMTs; the distribution of diagnoses for these patients was similar to those with opposite-sex BMTs. FISH interphase analysis correctly designated 149/153 (97.4%) as negative. All of the 4 false positive cases occurred in male recipients of like-sex BMT. One case had a 46, XY, -Y, +X karyotype, which led to a FISH result of 37.4% of cells with XX signals; the FISH results of the other 3 cases showed low levels of XX cells (4.6%, 1.6%, and 0.8%). FISH metaphase analyses designated 151/153 (98.7%) as negative. Both false positive cases were the same patients as those with discrepant FISH interphase analysis. One case had a 46, XY, -Y, +X karyotype, which led to a FISH result of 20% of cells with XX signals; the FISH results of the other case showed 7.1% XX cells.

The misclassification of a like-sex BMT recipient with an abnormal acquired karyotype demonstrates the importance of performing pre-BMT cytogenetic analysis in conjunction with FISH. The other 3 “false positive” cases by FISH had low levels of XX cells; both recipient and donor cells showed a 46,XY karyotype. Although no females with like-sex BMT cases were misclassified by FISH, low levels of donor/recipient cells by FISH should be interpreted with caution. All FISH results should be interpreted in conjunction with standard cytogenetic analysis and within the context of other relevant clinical information.

BIBLIOGRAPHY

1. Haas OA, Hinterberger W, Schmidmeier W, et al. Cytogenetic studies in bone marrow transplant recipients. *Blut.* 1986;53:29-38.
2. Walker H, Singer CRJ, Patterson J, et al. The significance of host haemopoietic cells detected by cytogenetic analysis of bone marrow from recipients of bone marrow transplants. *Br J Haematol.* 1986;62:385-391.
3. Lawler SD, Baker MC, Harris H, et al. Cytogenetic studies on recipients of allogenetic bone marrow using the sex chromosomes as markers of cellular origin. *Br J Haematol.* 1984;56:431-443.
4. Schattenberg A, DeWitte T, Salden M, et al. Mixed hematopoietic chimerism after transplantation with lymphocyte-depleted bone marrow is not associated with a higher incidence of relapse. *Blood.* 1989;73:1367-72.
5. Hook E. Exclusion of chromosomal mosaicism: Tables of 90%, 95%, and 99% confidence limits and comments on use. *American Journal of Human Genetics.* 1977;29:94-97.
6. U.S. Centers for Disease Control. *Morbidity and Mortality Weekly Review*. 1987;36(suppl. 2S):2S-18S.
7. Barch MJ, ed. *The AGT Cytogenetics Laboratory Manual*. 3rd ed. New York, NY: Raven Press, Ltd;1997.
8. Przepiorka D, Thomas ED, Durnam DM, et al. Use of a probe to repeat sequence of the Y chromosome for detection of host cells in peripheral blood of bone marrow transplant recipients. *Am J Clin Pathol.* 1991;95:201-206.
9. Alitalo T, Tihonen J, Hakola P, et al. Molecular characterization of a Y;15 translocation segregating in a family. *Hum Genet.* 1988;79:29-35.
10. Beyer WH. *CRC Handbook of Tables for Probability and Statistics*, Second Edition. CRC Press, Inc. Boca Raton, Florida, USA, 1968:219.
11. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. Washington, DC: US Government Printing Office, December 2009. [Also available online. *Type* > www.cdc.gov, *search* > BMBL5 > *look up* sections III and IV].
12. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
13. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
14. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization, 2004.
15. Erlecke J, Hartmann I, Hoffmann M, et al. Automated detection of residual cells after sex-mismatched stem-cell transplantation - evidence for presence of disease-marker negative residual cells. *Mol Cytogenet*. 2009;2:12.
16. Antin JH, Childs R, Filipovich AH, et al. Establishment of complete and mixed donor chimerism after allogeneic lymphohematopoietic transplantation: recommendations from a workshop at the 2001 Tandem Meetings of the International Bone Marrow Transplant Registry and the American Society of Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2001;7(9):473-485.

TECHNICAL ASSISTANCE

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