ANEUVYSION Multicolor DNA Probe Kit

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REF 05J38

30-608368/R5

ANEUVYSION **Multicolor DNA Probe Kit**
REF 05J38
30-608368/R5

Key to Symbols Used

	Manufacturer
REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
GTIN	Global Trade Item Number
Σ	Contains sufficient for <n> tests
	Temperature Limit
	Biological Risks
CONTROL –	Negative Control
CONTROL +	Positive Control
	Refer to **WARNINGS AND PRECAUTIONS** section
	Refer to **WARNINGS AND PRECAUTIONS** section
	Consult instructions for use
	Use By
EC REP	Authorized Representative in the European Community

ANEUVYSION MULTICOLOR DNA PROBE KIT

(Vysis CEP 18/X/Y-alpha satellite/LSI 13/21)

Part No. 32-161075, List No. 05J38-030;

Part No. 33-161075, List No. 05J38-010;

Part No. 35-161075, List No. 05J38-050

PROPRIETARY NAME

AneuVysion Multicolor DNA Probe Kit
(Vysis CEP 18, X, Y-alpha satellite, LSI 13 and 21)

COMMON OR USUAL NAME

Fluorescence in situ hybridization (FISH) reagents

INTENDED USE

The AneuVysion (Vysis CEP 18, X, Y-alpha satellite, LSI 13 and 21) Multicolor Probe Panel is intended to use CEP 18/X/Y probe to detect alpha satellite sequences in the centromere regions of chromosomes 18, X, and Y, and LSI 13/21 probe to detect the 13q14 region and the 21q22.13 to 21q22.2 region. The AneuVysion kit is indicated for identifying and enumerating chromosomes 13, 18, 21, X, and Y via FISH in metaphase cells and interphase nuclei obtained from amniotic fluid in subjects with presumed high risk pregnancies. It is not intended to be used as a stand alone assay for making clinical decisions.

FISH results are intended to be used as an aid in the diagnosis of numerical abnormalities of chromosomes 13, 18, 21, X and/or Y in conjunction with other information currently used in prenatal diagnosis, consistent with professional standards of practice.[1] This device is intended for use only with amniocyte cells; it is not intended for and has not been validated for use with other test matrices. This FISH assay will not detect the presence of structural chromosome abnormalities that can also result in birth defects. This FISH assay will be performed in cytogenetics laboratories.

SUMMARY AND EXPLANATION

Women at increased risk of carrying chromosomally abnormal fetuses are routinely offered amniocentesis with cytogenetic analysis of fetal cells. Indications for prenatal diagnosis include advanced maternal age, increased risk for fetal trisomy identified by maternal serum screening,[2,3] family history, maternal anxiety, and abnormal ultrasound results. Currently, fetal chromosome abnormalities are diagnosed by standard chromosome analysis (eg, karyotype), a method that detects chromosome 13, 18, 21, X, and Y aneuploidies with accuracy and is also capable of detecting visible structural abnormalities.

The FISH technique using chromosome-specific DNA probes may be used to assess the numerical autosomal aberrations in interphase cells in direct or cultured preparations of amniotic fluid samples.[4-17] Because sample processing for FISH interphase analysis does not require isolation and purification of DNA and/or radioactively labeled DNA probes, FISH results are typically obtained prior to receiving results from standard chromosome analysis. FISH results can be reported immediately; however, clinical decision making action based on FISH results should be carefully considered along with other medical evidence currently used in making prenatal diagnoses.[1,22]

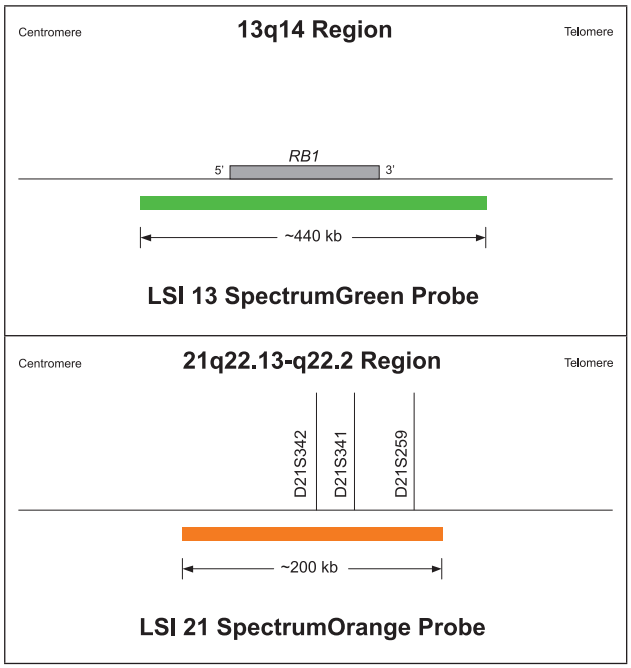
PRINCIPLES OF THE PROCEDURE

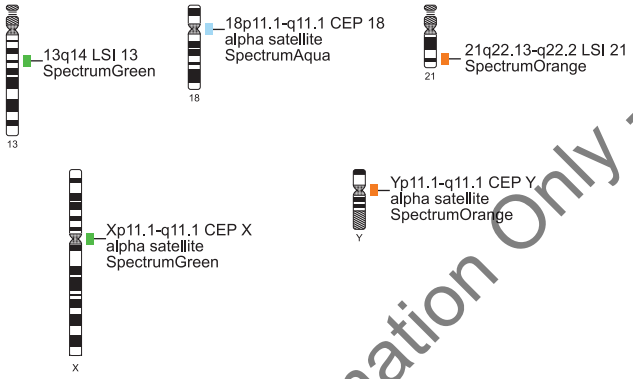
In situ hybridization is a technique that allows the visualization of specific nucleic acid sequences within a cellular preparation. Specifically, DNA FISH involves the precise annealing of a single stranded fluorescently labeled DNA probe to complementary target sequences. The hybridization of the probe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

Cells from amniotic fluid specimens are 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 18/X/Y and LSI 13/21 probes. 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 18/X/Y and LSI 13/21 DNA probes is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters allowing visualization of the intense orange, green, and aqua fluorescent signals and the blue counterstained chromosomes and nuclei. Enumeration of chromosomes 13, 18, 21, X, and Y is conducted by microscopic examination of interphase nuclei. The fluorescently stained chromosomes stand out brightly against the blue fluorescence of the nuclear DNA provided by the DAPI counterstain. The FISH procedure enables visual enumeration of chromosomes 13, 18, 21, X, and Y within the nuclei.

The CEP 18/X/Y probe is a mixture of directly labeled fluorescent DNA probes specific for the D18Z1, DXZ1, and DYZ3 regions of chromosomes 18, X ,and Y, respectively. The LSI 13/21 probe contains a mixture of unique DNA sequences that hybridize in the 13q14 region of chromosome 13, and unique DNA sequences complementary to the D21S259, D21S341, and D21S342 loci contained within the 21q22.13 to 21q22.2 region on the long arm of chromosome 21. The LSI 13 probe was created from a set of overlapping clones that contain the entire RB-1 gene, as well as regions extending beyond the gene on both sides.

The probe extends beyond the 180 kb RB-1 gene for 110 to 170 kb in the 5' direction and approximately 120 kb in the 3' direction; the entire probe is 410 to 470 kb. CEP 18/X/Y is an aqua, green, and orange tri-color probe mixture and LSI 13/21 is a green and orange dual color probe mixture. Both probe mixtures are pre-denatured in hybridization buffer for ease of use.





REAGENTS AND INSTRUMENTS

Materials Provided

AneuVysion Kit - 10 Assay (Part No. 33-161075, List No. 05J38-010)

1) LSI 13/21 DNA Probe: Low copy number *E. coli* vector
(The probe is pre-denatured)
Part No.: 33-171076
Quantity: 100 µL/1 vial (50 ng/µL)
Storage: –20°C (±5°C), protected from light
Composition: SpectrumGreen fluorophore labeled LSI 13 and SpectrumOrange labeled LSI 21 DNA probe pre-mixed with Blocking DNA and Hybridization Buffer (dextran sulfate, formamide, SSC).

2) CEP 18/X/Y DNA Probe: *E. coli* plasmid
(The probe is pre-denatured)
Part No.: 33-171075
Quantity: 100 µL/1 vial (25 ng/µL)
Storage: –20°C (±5°C), protected from light
Composition: SpectrumAqua fluorophore labeled alpha satellite DNA 18 probe, SpectrumGreen labeled alpha satellite DNA X probe and SpectrumOrange labeled alpha satellite DNA Y probe pre-mixed with Fluorophore labeled SpectrumGreen, SpectrumOrange and SpectrumAqua and non-labeled Blocking DNA and Hybridization Buffer (dextran sulfate, formamide, SSC).

3) DAPI II Counterstain
Part No.: 30-804861
Quantity: 600 µL/1 vial
Storage: –20°C (±5°C), protected from light
Composition: 125 ng/mL DAPI (4,6-diamidino-2-phenylindole) in phenylenediamine dihydrochloride, glycerol, and buffer.

4) NP-40
Part No.: 30-804820
Quantity: 4 mL/2 vials
Storage: –20 to 25°C
Composition: non-ionic detergent

5) 20X SSC
Part No.: 30-805850
Quantity: 66 g/1 container
Storage: –20 to 25°C
Composition: sodium chloride and sodium citrate

AneuVysion Kit - 30 Assay (Part No. 32-161075, List No. 05J38-030)

1) LSI 13/21 DNA Probe: Low copy number *E. coli* vector
(The probe is pre-denatured)
Part No.: 30-171078
Quantity: 300 µL/1 vial (50 ng/µL)
Storage: –20°C (±5°C), protected from light
Composition: SpectrumGreen fluorophore labeled LSI 13 and SpectrumOrange labeled LSI 21 DNA probe pre-mixed with Blocking DNA and Hybridization Buffer (dextran sulfate, formamide, SSC).

2) CEP 18/X/Y DNA Probe: *E. coli* plasmid
(The probe is pre-denatured)
Part No.: 30-171077
Quantity: 300 µL/1 vial (25 ng/µL)
Storage: –20°C (±5°C), protected from light
Composition: SpectrumAqua fluorophore labeled alpha satellite DNA 18 probe, SpectrumGreen labeled alpha satellite DNA X probe and SpectrumOrange labeled alpha satellite DNA Y probe pre-mixed with Fluorophore labeled SpectrumGreen, SpectrumOrange and SpectrumAqua and non-labeled Blocking DNA and Hybridization Buffer (dextran sulfate, formamide, SSC).

3) DAPI II Counterstain
Part No.: 30-804861
Quantity: 1200 µL/2 vials
Storage: –20°C (±5°C), protected from light
Composition: 125 ng/mL DAPI (4,6-diamidino-2-phenylindole) in phenylenediamine dihydrochloride, glycerol, and buffer.

4) NP-40
Part No.: 30-804820
Quantity: 4 mL/2 vials
Storage: –20°C to 25°C
Composition: non-ionic detergent

5) 20X SSC
Part No.: 30-805850
Quantity: 66 g/1 container
Storage: –20°C to 25°C
Composition: sodium chloride and sodium citrate

AneuVysion Kit - 50 Assay (Part No. 35-161075, List No. 05J38-050)

1) LSI 13/21 DNA Probe: Low copy number *E. coli* vector
(The probe is pre-denatured)
Part No.: 35-171078
Quantity: 500 µL/1 vial (50 ng/µL)
Storage: –20°C (±5°C), protected from light
Composition: SpectrumGreen fluorophore labeled LSI 13 and SpectrumOrange labeled LSI 21 DNA probe pre-mixed with Blocking DNA and Hybridization Buffer (dextran sulfate, formamide, SSC).

2) CEP 18/X/Y DNA Probe: *E. coli* plasmid
(The probe is pre-denatured)

Part No.: 35-171077
Quantity: 500 µL/1 vial (25 ng/µL)
Storage: –20°C (±5°C), protected from light
Composition: SpectrumAqua fluorophore labeled alpha satellite DNA 18 probe, SpectrumGreen labeled alpha satellite DNA X probe and SpectrumOrange labeled alpha satellite DNA Y probe pre-mixed with Fluorophore labeled SpectrumGreen, SpectrumOrange and SpectrumAqua and non-labeled Blocking DNA and Hybridization Buffer (dextran sulfate, formamide, SSC).

3) DAPI II Counterstain

Part No.: 30-804861
Quantity: 1200 µL/2 vials
Storage: –20°C (±5°C), protected from light
Composition: 125 ng/mL DAPI (4,6-diamidino-2-phenylindole) in phenylenediamine dihydrochloride, glycerol, and buffer.

4) NP-40

Part No.: 30-804820
Quantity: 4 mL/2 vials
Storage: –20°C to 25°C
Composition: non-ionic detergent

5) 20X SSC

Part No.: 30-805850
Quantity: 66 g/1 container
Storage: –20°C to 25°C
Composition: sodium chloride and sodium citrate

Storage and Handling

Store the unopened AneuVysion Kit as a unit at –20°C (±5°C), protected from light and humidity. The 20X SSC salts and NP-40 may be stored separately at room temperature. Expiration dates for each of the components are indicated on the individual component labels. These storage conditions apply to both opened and unopened components.

Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

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

- ProbeChek Normal Male Amniocyte Control Slides, Part No. 30-805010, List No. 05J39-005. Fixed biological specimen derived from normal human male amniocytes applied to glass microscope slides. Quantity: 5 slides (10 target areas). Store the control slides at –20°C (±5°C) in a sealed container with desiccant to protect them from humidity.
- ProbeChek Prenatal Positive Control Slides, Part No. 30-805017, List No. 05J36-005. Fixed biological specimen derived from human fibroblast cells applied to glass microscope slides. Quantity: 5 slides (10 target areas). Store the control slides at –20°C (±5°C) in a sealed container with desiccant to protect them from humidity.
- Formamide, ultra-pure grade.
- Ethanol (100%). Store at room temperature.
- Concentrated (12N) hydrochloric acid (HCl)
- 1N sodium hydroxide (NaOH)
- Purified water (distilled or deionized). Store at room temperature.
- Fixative (3:1 methanol:acetic acid). Prepare fresh daily.
- Drierite desiccant
- 1X Trypsin/EDTA (0.05% Trypsin, 0.53 mM EDTA-4Na in Hanks' Balanced Salt Solution without $CaCl_2$, $MgCl_2 \cdot 6H_2O$, and $MgSO_4 \cdot 7H_2O$)
- 0.56% potassium chloride (KCl)
- Reagents for optional pretreatment of late gestational amniotic fluid cells:
  - Pepsin working solution (2.5 mg pepsin added to 50 mL of 0.01 N HCl)
  - Post fixation solution (1.3 mL of 37% formaldehyde, 0.23g $MgCl_2$, and 48.7 mL of PBS)

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 clean tips
- Polypropylene microcentrifuge tubes (0.5 mL or 1.5 mL)
- Timer
- Magnetic stirrer
- Vortex mixer
- Microcentrifuge
- Graduated cylinder
- Water baths (37±1°C, 67±2°C, and 73±1°C)
- Air incubator (37±2°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 auto fluorescence and specifically for use in fluorescence microscopy.

Filters: Single and multi-bandpass fluorescence microscope filter sets optimized for use with the AneuVysion kit are available from Abbott Molecular Inc. for most microscope models. Orange, Green, and Aqua single bandpass, and triple bandpass DAPI/Green/Orange filter sets are recommended for the AneuVysion kit. The triple bandpass filter configuration allows the simultaneous excitation and emission of the SpectrumGreen, SpectrumOrange, and DAPI fluorophores. The SpectrumOrange signals will be pinkish orange; the SpectrumGreen signals will be greenish-yellow. All of the other DNA will fluoresce blue with the DAPI stain. SpectrumAqua signals will be aqua-blue through the single bandpass Aqua filter set.

Preparation of Working Reagent Solutions

20X SSC (pH 5.3)

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 using a pH meter. Verify the 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. Add 70 mL of each solution to a Coplin jar and maintain at room temperature. Store unused dilutions in a covered container at room temperature for up to 6 months. Solutions used in the assay may be used for 1 week unless evaporation occurs or the solution becomes diluted due to excessive use.

0.3% NP-40 in 0.4X SSC Wash Solution

To prepare, add together:

950 mL	Purified water
20 mL	20X SSC pH 5.3
3 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. 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 Wash Solution

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.

NOTE: Additional reagents are needed for the specimen processing and slide preparation procedures. Refer to the specific procedure section for details.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

1. FOR IN VITRO DIAGNOSTIC USE
2. All biological specimens should be treated as if capable of transmitting infectious agents. ProbeChek Control Slides are manufactured from human cultured cells that have been fixed in a solution of methanol:acetic acid (3:1, v:v). *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 U.S. Centers for Disease Control and Prevention.*[18]
3. Hybridization conditions may be adversely affected by the use of reagents other than those provided or recommended by Abbott Molecular Inc.
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 that do not require light for manipulation (incubation periods, washes, etc.) in subdued light.
6. Probe mixtures 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, local and state guidelines for hazardous disposal.

BioSafety Statement for Kit Component:

- **LSI 13/21 DNA Probe**
- **CEP 18/X/Y 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,[25] OSHA Standards on Bloodborne Pathogens,[26] CLSI Document M29-A3,[27] and other appropriate biosafety practices.[28] 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.[25]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[28]

LSI 13/21 DNA Probe and CEP 18/X/Y DNA Probe

Danger

Hazard-determining components of labeling: Formamide

H360	May damage fertility or the unborn child.
P201	Obtain special instructions before use.
P281	Use personal protective equipment as required.
P303+ P361+P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. rinse skin with water/shower.
P308+P313	IF exposed or concerned: Get medical advice/ attention.
P403+P235	Store in a well-ventilated place. Keep cool.
P501	This material and its container must be disposed of in a safe way.

NP-40

Danger

Hazard-determining components of labeling: Ethoxylated octyl phenol

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P280	Wear protective gloves/protective clothing/ eye protection.
P264	Wash hands thoroughly after handling.
P305+ P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P302+P352	IF ON SKIN: Wash with plenty of water.
P337+P313	If eye irritation persists: Get medical advice/ attention.
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.

NOTE: Material Safety Data Sheets (MSDS) for all reagents provided in the kit are available upon request from the Abbott Molecular Technical Services Department.

SPECIMEN COLLECTION, PROCESSING, STORAGE, AND SLIDE PREPARATION

Specimen Collection and Processing

The AneuVysion kit is designed for use on both uncultured and cultured amniocytes. Amniotic fluid collections should be performed according to the laboratory's institution guidelines. The ACT Manual contains a number of recommendations for collection and culturing of specimens.[19]

The amniotic fluid sample must be collected in a manner that minimizes the risk of maternal cell contamination.[20] To reduce maternal contamination, ACOG recommends discarding the first 1 to 2 mL of the amniocentesis specimen.[22] For collection of amniotic fluid, the ACT recommends using syringes with sealant shown not to affect cell growth adversely and containers specifically marked as approved for cell culture. Prepackaged amniocentesis trays are also recommended.[19] The minimum volume of amniotic fluid required for this FISH assay is 2 to 5 mL, depending on gestational age. The user must carefully weigh the risks and benefits of utilizing aliquots of the specimen for FISH when the specimen volume does not meet the minimum requirements for both FISH and standard cytogenetics. See **Table 1**.

According to the ACT Manual, the time from collection of the amniotic fluid to culturing should be as brief as possible (immediately to overnight) and care must be taken to avoid extremes in temperature. Specimens should never be iced or frozen.[19]

Bloody 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. Amniotic fluid samples that appear bloody or brownish in color should not be used, since these samples may contain maternal blood.[20]

Table 1. Recommended Volumes of Amniotic Fluid Withdrawn for FISH and Standard Chromosome Analysis

Gestation Week(s)	Total Volume Drawn (mL)	Volume (mL) Reserved for Standard Chromosome Analysis	Minimum Volume (mL) for FISH
11 to 15	14	12	2
16	16	13	3
17	16	13	3
18	18	14	4
19	18	14	4
20 to 27	25+	15+	5+

Slide Preparation from Uncultured Amniotic Fluid

The following method may be used for preparing slides from uncultured amniotic fluid specimens.

1. Centrifuge 2 to 5 mL of whole amniotic fluid (AF) specimen for 5 minutes at 1,000 RPM. The specimen should not appear bloody or brown.
2. Resuspend the pellet in 2 to 5 mL of 1X trypsin/EDTA (0.05% Trypsin, 0.53 mM EDTA·4Na in Hanks' Balanced Salt Solution without $CaCl_2$, $MgCl_2 \cdot 6H_2O$, and $MgSO_4 \cdot 7H_2O$) and incubate in a 37±1˚C water bath for at least 15 minutes.
3. Centrifuge the suspension for 5 minutes at 1,000 RPM.
4. Resuspend the pellet in 2 to 5 mL of 0.56% KCl and incubate for 20 minutes in a 37±1˚C water bath.
5. Add 0.8 to 2 mL of fixative (3:1 methanol:glacial acetic acid) to the cells/hypotonic solution and vortex gently.
6. Centrifuge the suspension for 5 minutes at 1,000 RPM and resuspend the pellet in 1 mL fresh fixative. Store fixed specimens at 4˚C for at least 30 minutes or until ready to perform FISH. For long term storage, store fixed specimens at – 20˚C (±5˚C) in fixative.
7. Before placing cells on slides, adjust volume of cell suspension according to size of cell pellet. If needed, particularly after a prolonged storage (> 1 month), wash pellets with fixative before slide preparation.
8. To prepare slides for FISH, drop the cell suspension directly onto 1 or 2 cold, glass slides, making 2 hybridization areas (15 to 25 µL of cell suspension per area).
9. Proceed to **Pretreatment for Uncultured Amniotic Fluid Cells**. If the optional pretreatment procedure will not be used, age the specimen slides in 2X SSC for 30 minutes to 1 hour at 37˚C OR at room temperature for 24 hours, with the slide box uncovered, before hybridization or storage.

Pretreatment For Uncultured Amniotic Fluid Cells (Optional)

The following method may be used for pretreating specimen slides prepared from uncultured amniotic fluid specimens. This procedure is recommended in order to achieve optimum FISH results. Pretreatment is especially beneficial for late gestational age specimens and specimens to be hybridized immediately following slide preparation.

1. Place slide(s) prepared from uncultured amniocytes, in 2X SSC for 1 hour at 37±1˚C.
2. Place slide(s) in freshly made pepsin working solution (2.5 mg pepsin added to 50 mL of 0.01 N HCl) for 13 minutes at 37±1˚C.
3. Rinse slide(s) in phosphate buffered saline (PBS) at room temperature for 5 minutes.
4. Place slide(s) in post fixation solution (0.95% formaldehyde: 1.3 mL of 37% formaldehyde, 0.23g $MgCl_2$ and 48.7 mL of PBS. Store at 4˚C. Use within 1 month.) for 5 minutes at room temperature.
5. Rinse slide(s) in PBS at room temperature for 5 minutes.
6. Dry slide(s). To speed drying, use a cotton-plugged Pasteur pipet to direct compressed air flow onto the slide.
7. Immerse slide(s) in 70% ethanol at room temperature. Allow the slide(s) to stand in the ethanol wash for 1 minute.
8. Remove the slide(s) from 70% ethanol. Repeat step 7 with 85% ethanol, followed by 100% ethanol.
9. Denature slides(s) according to the instructions in this package insert.

Slide Preparation for Fixed Cell Pellets from Cultured Specimens

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, not allowing the water to touch the bottom of the slides.
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 racks 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 nuclei per field under low power (10X objective). A minimum of approximately 100 cells per low power field is recommended for optimum assay results. Adjust the cell 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. Age the specimen slides in 2X SSC for 30 minutes to 1 hour at 37±1˚C OR 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 containing approximately 1 tablespoon of Drierite. Store at – 20˚C (±5˚C) prior to hybridization.

Storage of amniocyte specimens on prepared slides for FISH assay at a later date is not recommended. Long term storage of prepared slides could lead to poor signal quality. When the amniocytes are stored as pellets as recommended, FISH assay on the freshly dropped slides invariably leads to better signal quality. In routine application, the FISH assay is generally performed immediately following slide preparation.

ASSAY PROCEDURE: FISH PROCEDURE SUMMARY

Denaturation of Specimen DNA:

1. Prewarm the humidified hybridization chamber (an airtight container with a piece of damp blotting paper or paper towel approximately 1 in. x 3 in. taped to the side of the container) to 37±2˚C by placing it in the 37±2˚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. Verify that the pH of the denaturing solution is 7.0 to 8.0 before each use. 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 10 µL of CEP 18/X/Y probe mix to one hybridization area on the slide(s) and 10 µL of LSI 13/21 probe mix to the other hybridization area on the slide(s). Immediately place a 22 mm × 22 mm 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. Seal coverslip with rubber cement as follows: Draw the rubber cement into a 5 mL syringe. Eject a small amount of rubber cement around the periphery of the coverslip overlapping the coverslip and the slide, forming a seal around the coverslip.
3. Place the slide into the pre-warmed 37 ± 2˚C hybridization chamber, cover the chamber with a tight lid and incubate at 37 ± 2˚C for 6 to 24 hours.

Post-hybridization Washes

1. Add 0.4X SSC/0.3% NP-40 to a Coplin jar. Prewarm the 0.4X SSC/0.3% NP-40 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. *The temperature of the wash solution must return to 73 ± 1˚C before washing each batch.*
2. Add 2X SSC/0.1% NP-40 to a second Coplin jar and place at room temperature. Discard both wash solutions after 1 day of use.
3. Remove rubber cement seal and coverslips. Immediately place the slide into the Coplin jar containing 0.4X SSC/0.3% NP-40 at 73 ± 1˚C. Agitate the slide for approximately 3 seconds. Repeat for the other slides, then incubate 2 minutes. Do not place more than 4 slides in the wash at one time. *If more than 4 slides are to be washed, verify that the temperature of the wash solution is 73 ± 1˚C before each use.*
   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.
4. 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.
5. Allow the slide to air-dry in the dark (a closed drawer or a shelf inside a closed cabinet is sufficient.)
6. Apply 10 µL of DAPI II counterstain to each 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 (±5˚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 (±5˚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 to and illuminate only the corresponding target DNA on the chromosome. On cultured specimens, metaphase spreads may be evaluated to identify any cross-hybridization to non-target sequences. At least 98% of cells should show one or more signals for acceptable hybridization (see guidelines for signal enumeration below).

If any of the above features are unsatisfactory, consult the **Troubleshooting Guide**, **Table 2**, and process a fresh slide.

Selection of best 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 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 containing 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 50 nuclei are enumerated and analyzed for each target. When mosaicism (10 to 60% aneuploid cells) is expected, 200 nuclei per target should be enumerated. A target with less than 50 evaluable nuclei should be either supplemented with an additional slide, or considered an uninformative case.

Interphase Enumeration

Enumerate the fluorescent signals in each evaluable interphase nucleus using a 40X or 63X objective. Follow the signal counting guidelines in **Figures 1** and **2**. 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.
- For CEP 18, LSI 13 and LSI 21, enumerate the number of nuclei with 0, 1, 2, 3, 4, or > 4 signals. Count only those nuclei with 1 or more FISH signal of any color. If the accuracy of enumeration is in doubt, repeat the enumeration in another area of the slide.
- For CEP X/Y, enumerate the number of nuclei with 0, 1, 2, 3, 4, or > 4 signals (for both orange and green signals) and record the counts in a 2-way table, and then calculate the percentage of nuclei with X, Y, XY, XX, XXY, XYY, XXX, and others. Count only those nuclei with 1 or more FISH signal of any 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.

Table 2. 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 and replace lens
	• Dirty or broken mirror in lamp housing	• Clean or replace mirror
	• Inappropriate objectives	• Use recommended objectives
	• Hybridization conditions inappropriate	• Check temperature of 73±1°C water bath
	• Wash conditions inappropriate	• Check temperature of 37±2°C incubator
		• Check temperature 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 (±5°C) in darkness
• Low signal specificity	• Hybridization conditions inappropriate	• Check temperature of 37±2°C incubator
	• Wash temperature too low	• Maintain wash temperature at 73±1°C
• High slide background	• Slides 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 min each in 50% formamide/2X SSC pH 7.5 to 8.0, at 46±1°C. 2. Wash slide(s) 1X for 10 min in 2X SSC, at 46±1°C. 3. Wash slide(s) 1X for 5 min 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 2. Troubleshooting Guide (continued)

Problem	Probable Cause	Solution
• "Distorted" chromosome morphology (Cultured specimens)	• 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
	• Denatured specimen slide too long	• Reduce denaturation time in 1 minute increments
• 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

Table 3. Signal Distribution for CEP 18, X, and Y (alpha), and LSI 13 and 21 as determined using the ProbeChek Normal Male Amniocyte Control Slide

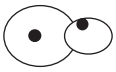
	%0 signals	%1 signal	%2 signals	%3 signals	%4 signals	%>4 signals	
CEP 18	0.0-2.0	0.0-2.1	93.0-100	0.0-2.1	0.0-4.0	0.0-1.0	
LSI 13	0.0-2.0	0.0-2.1	93.0-100	0.0-2.1	0.0-5.0	0.0-1.0	
LSI 21	0.0-2.0	0.0-2.1	93.0-100	0.0-2.1	0.0-5.0	0.0-1.0	
	%X	**%Y**	**%XX**	**%XY**	**%XXX**	**%XXY**	**%XYY**
CEP X/Y	0.0-2.0	0.0-2.0	0.0-2.0	93.0-100	0.0-2.0	0.0-2.0	0.0-2.0

Table 4. Signal Distribution for CEP 18, X, and Y (alpha), and LSI 13 and 21 as determined using the ProbeChek Prenatal Positive Control Slide

	%0 signals	%1 signal	%2 signals	%3 signals	%4 signals	%>4 signals
CEP 18	0.0-1.0	0.0-2.0	0.0-10.0	86.0-100.0	0.0-2.0	0.0-1.0
CEP X	0.0-1.0	0.0-1.0	80.0-100.0	0.0-14.0	0.0-5.0	0.0-1.0
CEP Y	0.0-2.0	96.0-100.0	0.0-3.0	0.0-1.0	0.0-1.0	0.0-1.0
LSI 13	0.0-1.0	0.0-1.0	0.0-5.0	94.0-100.0	0.0-1.0	0.0-1.0
LSI 21	0.0-1.0	0.0-1.0	0.0-2.0	95.0-100.0	0.0-2.0	0.0-1.0

Figure 1. Single Color Signal Counting Guide

(LSI 21 SpectrumOrange: Use Orange single pass filter for enumeration, LSI 13 SpectrumGreen: Use Green single pass filter for enumeration and CEP 18 SpectrumAqua: Use Aqua single pass filter for enumeration)

1		Don't count, skip over. This could be 2 cells with 1 signal each or 1 twisted nucleus.
2		Count as 2 signals: 1 is very compact, the other is diffuse.
3		Don't count, skip over. Observer cannot determine which cell contains the signals.
4		Count as 2 signals. One signal is split.
5		Count as 3 signals.
6		Count as 4 signals.
7		Count as 3 signals. One is split.

Quality Control

Use of Control Slides - Positive Control (human fibroblast cell line) and Negative Control (normal male human amniocytes) 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. ProbeChek control slides are recommended.

Slide adequacy and signal enumeration should be assessed using the criteria described above in the signal enumeration section. Perform signal enumeration on 50 to 200 nuclei. For CEP 18, LSI 13, and LSI 21, enumerate the number of nuclei with 0, 1, 2, 3, 4, or >4 signals. Count only those nuclei with 1 or more FISH signals of any color. For CEP X/Y, enumerate the number of nuclei with 0, 1, 2, 3, 4, or >4 signals (for both orange and green signals) and record the counts in a 2-way table, and then calculate the percentage of nuclei with X, Y, XY, XX, XXY, XYY, XXX, and others. Again, count only those nuclei with one or more FISH signals of any color. Record the number of nuclei with 1, 2, 3, 4 and >4 CEP or LSI DNA probe signals, and then calculate the percentage of nuclei with 3 signals among all cells with at least 1 hybridized signal. The criteria for slide adequacy must be satisfied and the signal enumeration results should be within the specifications described in **Tables 3** and **4** for acceptable test performance.

If control slides fail to meet the slide acceptance criteria, the assay may not have been performed properly or the AneuVysion 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 2** for probable causes and the actions needed to correct the problems.

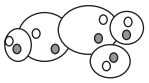
If control slides meet the acceptance criteria but the enumeration values are outside the specified range, the enumeration may not have been performed correctly and an independent, repeat assessment of the same slide may be appropriate.

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.

Figure 2. Dual Color Signal Counting Guide

(CEP X SpectrumGreen/CEP Y SpectrumOrange: Use DAPI/ Orange/Green filter for enumeration, and use specific single pass for confirmation)

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 one green signal.
8		Count as 4 orange signals.

TEST INTERPRETATION

Results on enumeration of 50 interphase nuclei per target are reported as the number and percentage of nuclei with 1, 2, 3, 4, and >4 signals for CEP 18, LSI 13, and LSI 21 and as the number and percentage of nuclei with X, Y, XX, XY, XXY, XYY, XXX, and others, for CEP X/Y. Our clinical study found that specimens with true aneuploidy by standard chromosome analysis, showed >60% aneuploid nuclei with FISH analysis; and those that were cytogenetically euploid all showed <10% aneuploid nuclei with FISH. Values near the 10% cut-off should be interpreted with caution since low levels of mosaicism cannot be ruled out. Standard cytogenetic procedures should be followed to distinguish between true mosaicism and pseudomosaicism. The user should verify these expected values or establish their own.

Discrepancies in results among test methods may be due to inaccurate results from one or more of the test methods, differences in analytical sensitivity/specificity among the methods, actual differences in aneuploidy status among the cell populations assessed with the different methods (eg, cycling metaphase cells vs. non-cycling interphase cells), among others. Repeat FISH (concurrently run with QC material) and/or repeat standard chromosome 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 other clinical findings, a consultation between the cytogeneticist and the treating physician is warranted.

LIMITATIONS

1. The AneuVysion kit has been characterized only for identifying targeted regions of chromosomes X, Y, 18, 13, and 21 in interphase nuclei from uncultured and cultured amniocyte specimens.
2. The clinical interpretation of any test result(s) should be made in conjunction with other diagnostic laboratory test results and should be evaluated within the context of the patient's medical history and current risk factors. Clinical decision making should be based on information from FISH and at least 1 of the following results: confirmatory standard chromosome analysis or consistent clinical information, such as an abnormal ultrsound finding or a positive screening test result for Down syndrome or trisomy 18.[1,22-24]
3. FISH assay results may not be informative if the specimen quality and/or specimen slide preparation is inadequate.
4. This assay will not detect the presence of structural abnormalities frequently associated with birth defects. The frequency of these occurrences may be population and gestational age dependent.
5. This assay should not be performed on amniocyte specimens with moderate to severe maternal cell contamination. FISH test results on amniocyte specimens with mild maternal cell contamination should be interpreted with caution.

6. No irreversible therapeutic action should be initiated based on a positive FISH assay alone. Positive results should be further characterized using standard chromosome analysis to determine the mutational mechanism accounting for the abnormality detected by FISH. This information may aid in counseling for the risk that the detected abnormality may occur in future pregnancies.[1,22]
7. Physicians, counselors, and other healthcare providers should understand the risk of abnormalities that the test is not designed to detect. The patient should be informed that there is still a very small risk of low level mosaicism, cryptic translocations, or other undetectable events that may not be demonstrated by FISH or standard cytogenetics. Additionally, there is a very small risk that some individuals carry a genetic polymorphism that may affect the intensity, presence or absence of the probe signal that may result in a missed diagnosis.[17]
8. When the specimen volume is not sufficient to meet the minimum requirements for processing both FISH and standard cytogenetic procedures, the user must carefully weigh the risks and benefits of utilizing any material for FISH. Consultation between the laboratory geneticist and/or genetic counselor and the patient's physician may aid in clarifying what information is desired, and which testing method should be used.[1]
9. Technologists performing the FISH signal enumeration must be capable of visually distinguishing between the orange, green and aqua signals.
10. Although the probe for enumerating chromosome 13 spans the Rb1 locus, this probe has not been validated for detecting mutations associated with retinoblastoma. In rare cases, the Rb1 locus may be deleted; this could complicate interpretation of FISH test results.

EXPECTED VALUES

FISH interphase signal enumeration was performed on euploid and aneuploid amniotic fluid specimens to assess the expected percentage of cells with 0, 1, 2, 3, and ≥4 signals (CEP 18, LSI 13, and LSI 21) and with X, Y, XX, XY, XXY, XYY, XXX, and others (CEP X/Y).

Values Among Normal Amniotic Fluid Specimens

FISH interphase analysis was performed on both cultured and uncultured amniotic fluid specimens from 504 normal subjects (429 uncultured and 75 cultured), collected at 31 sites, to determine the expected values and to verify the cutoff point. There was no significant difference in the signal distribution between uncultured and cultured specimens among these normal cases. Fifty nuclei were enumerated per probe; the distribution of signals for these subjects is summarized in **Tables 5** and **6**.

Table 5. Distribution of Percentage of Cells With Chromosome 13, 18, and 21 Signals in Normal Amniotic Fluid Specimens

		Percent of Cells With				
Chromosome	**Statistics**	**0-signal**	**1-signal**	**2-signals**	**3-signals**	**4-signals**
13	Mean	0.59	2.71	95.6	0.70	0.04
	S.D.	2.73	3.17	5.17	1.43	0.37
18	Mean	0.38	3.75	94.4	0.99	0.01
	S.D.	1.94	4.01	5.47	1.87	0.20
21	Mean	0.34	2.93	94.6	1.57	0.10
	S.D.	2.06	3.29	5.33	2.18	0.77

For CEP X/Y, the percentage of cells with X, Y, XX, XY, XXY, XYY, XXX, and others are categories of interest for assessing expected values, among which the percentage of cells with XX in females and XY in males are the critical categories in cytogenetically normal amniotic fluid specimens. The mean (±S.D.) percentages of cells with specific X/Y signals are shown in **Table 6**.

Table 6. Distribution of Percentage of Cells with X and Y Signals in 376 Female and 445 Male Normal Amniotic Fluid Specimens

Genetic Sex	**Statistics**	**X0**	**0Y**	**XX**	**XY**	**XXX**	**XXY**	**XYY**
46, XX	Mean	2.54	0.002	96.0	0.02	0.60	0.09	0.00
	S.D.	2.62	0.052	4.46	0.24	1.29	0.57	0.00
46, XY	Mean	0.99	0.36	0.30	96.9	0.00	0.35	0.39
	S.D.	2.09	1.44	1.66	4.39	0.00	1.07	1.23

In cytogenetically normal amniotic fluid specimens, the percentage of bi-signaled and tri-signaled cells are 2 critical categories for assessing expected values for CEP 18, LSI 13, and LSI 21. The mean (±S.D.) percentages of bi-signaled and tri-signaled nuclei were 95.60% (±5.17%) and 0.70% (±1.43%), respectively, for chromosome 13; 94.44% (±5.47%) and 0.99% (±1.87%), respectively, for chromosome 18; and 94.63% (±5.33%) and 1.57% (±2.18%), respectively, for chromosome 21.

For CEP X/Y, the percentage of cells with XY nuclei in males and XX nuclei in females are 2 critical categories for assessing expected values. The mean (±S.D.) percentages of cells with XY nuclei in normal males was 96.91% (±4.39%). The mean (±S.D.) percentages of cells with XX nuclei in normal females was 96.01% (±4.46%).

Values Among Aneusomic Amniotic Fluid Specimens

In cytogenetically abnormal amniotic fluid specimens, the critical category for LSI 13, CEP 18, and LSI 21 is the percentage of cells with three signals (% tri-signaled nuclei). The critical category for CEP X/Y is the percentage of cells with signals other than XY nuclei in males and XX nuclei in females. These critical categories for chromosomes 13, 18, 21, X, and Y will be called aneuploid cells.

There were 854 (821 true aneuploid and 33 triploid) aneuploid specimens, by standard chromosome analysis in the pivotal study. Of these 854 specimens, 455 were cultured and 399 were uncultured. The distribution of % aneuploid cells showed 6 specimens (0.7%) with 63 to 69%, 25 (3.0%) with 70 to 79%, 73 (8.9%) with 80 to 89%, and 717 (87.3%) with 90 to 100%. The percentage of aneuploid nuclei was not significantly different between the cultured and uncultured specimens for all types of aneusomy.

The mean and S.D. of % aneuploid cells for these subjects is presented by aneuploidy in **Table 7**.

Table 7. Mean (±S.D.) Percentage of Aneuploid Cells by Aneuploidy

	% Aneuploid Cells		
Aneuploidy	**n**	**Mean**	**S.D.**
+13	75	91.52	5.91
+18	192	88.87	7.10
+21	322	91.51	7.03
triploid-XXY	14	94.64	9.28
triploid-XXX	19	93.21	6.15
X	107	96.96	5.11
XXX	44	93.56	7.61
XXY	57	95.84	5.44
XYY	24	94.96	5.51

Verification of Cut-off Point

In the pivotal study, the published cutoff point of 60% was verified.[14] Among the 504 euploid specimens, the largest percentage of aneuploid cells was 10%, and among the 854 aneuploid samples, the smallest percentage of aneuploid cells by FISH assay was 63%. There was a wide gap between the largest value among euploid specimens and the smallest value among aneuploid specimens, reducing the chance of misclassification, with 60% as the cutoff point.

Values Among Mosaic Amniotic Fluid Specimens

In 62 cytogenetically mosaic amniotic fluid specimens, the percentage of aneuploid cells as detected by FISH assay ranged from 2% to 94%. The % aneuploid nuclei, as measured by FISH and standard cytogenetics, are correlated ($r = 0.76$), but not perfectly. It is yet to be determined which of the 2 measurements is better correlated with phenotypic expression.

Values Among Pseudomosaic Amniotic Fluid Specimens

In 76 cytogenetically pseudomosaic specimens, the % bi-signaled nuclei by FISH assay ranged from 86% to 100% and the % aneuploid cell ranged from 0% to 4.8%.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity and Specificity

Hybridization Efficiency

In the pivotal study among the human amniotic fluid specimens, the average percentage of cells with no hybridization signal was 0.42% for LSI 13, CEP 18, and LSI 21. The average percentage of cells with only one hybridization Y signal was 0.06% for CEP X/Y. Thus, <2% cells with no or only 1 signal for each probe is a realistic standard of acceptance.

Analytical Sensitivity

The analytical sensitivity of the AneuVysion kit probes was tested in the reproducibility study described below. In that study, the 100% XY (or 0% X0) specimen was estimated with a mean of 0.04% (±0.2%), and the 10% X specimen was estimated with a mean of 9.10% (±1.79%) X-signaled nuclei. The upper 95% CI was 0.43 for the 0% 45, X specimen and the lower 95% CI for the 10% X specimen was 5.59%. Thus, the limit of detection for the AneuVysion kit in interphase cells is estimated to be 3%.

Analytical Specificity

Locus specificity studies were performed with metaphase spreads according to standard Abbott Molecular Quality Control (QC) protocols. A total of 705 metaphase spreads were examined sequentially by G-banding to identify chromosomes 13, 18, 21, X, and Y, followed by FISH. No cross-hybridization to other chromosome loci was observed in any of the 705 cells examined; hybridization was limited to the target regions of chromosomes 13, 18, 21, X, and Y.

Stringency studies were also performed, according to standard Abbott Molecular QC protocols, on lymphocyte and amniocyte specimens to determine the temperatures at which assay failure occurs; to determine the cross-hybridization patterns under reduced stringency conditions; and to estimate the hybridization efficiency under normal and stressed stringency conditions. All assays performed within the recommended temperature range (ie, at 72 and 74˚C) passed the quality evaluation and achieved the highest overall rating among all test points. Uncultured amniocyte specimens performed well under reduced stringency conditions and achieved passing scores even when processed at 62˚C. Cross hybridization of the CEP 18 probe was observed among cultured amniocytes at lower wash temperatures below 72˚C. Cross-hybridization was confirmed by detailed image analysis performed by inverting the DAPI banding pattern on metaphases, and was observed specifically at the centromere of chromosome pair 20, but not on any other chromosome or chromosome pair. Wash temperatures above 74˚C resulted in decreased signal intensity, but specificity, cross-hybridization and background ratings remained acceptable in evaluable targets.

Reproducibility

To assess the reproducibility of the AneuVysion assay, analyses for the percentage of aneuploid cells were assessed for inter-site, inter-lot, inter-day, and inter-observer reproducibility. One normal (46,XY) plus three cultured human amniocyte mixtures with known percentages of mosaicism [45,X(10%)/46,XX(90%), 45,X(17%)/46,XX(47%)/47, XXX(36%), and 47,XY,+21(50%)/46,XY(50%)] were evaluated for the percentage of aneuploid cells according to the instructions for signal enumeration in the package insert. The overall hybridization success rate was 85% (102/120) on the first try. Hybridizations of the 18 replacement slides (all at 1 site) were all successful.

Using ANOVA, no statistically significant variations were observed in any of the study parameters. The mean, standard deviation, and percent CV of the observed percentage of aneuploid cells are shown in **Tables 8** through **12**.

Table 8. Precision of % Aneuploid Cells by Level of Mosaicism

Specimen	N	Summary Statistics	%X	%XX	%XXX	%XY	+21	%2-sig CH-21	%2-sig CH-18	%2-sig CH-13
100% XY	24	Mean	0.04	0.04	0.00	98.88	0.79	96.7	94.60	97.1
		S.D.	0.20	0.20	0.00	1.88	0.79	2.34	3.37	2.35
		C.V. (%)	—	—	—	1.9	100	2.4	3.6	2.4
10% X/ 90% XX	24	Mean	9.10	88.17	0.85	0.00	1.25	94.80	94.90	95.60
		S.D.	1.79	2.88	1.08	0.00	0.97	4.35	4.16	4.14
		C.V. (%)	19.7	3.30	—	—	77.6	4.6	4.4	4.3
17% X/ 47% XX/ 36% XXX	24	Mean	19.48	42.56	36.68	0.00	1.16	96.10	95.70	97.30
		S.D.	4.14	4.88	3.58	0.00	0.98	3.11	3.37	2.09
		C.V. (%)	21.3	11.5	9.8	—	84.5	3.2	3.5	2.1
50% XY+21/ 50% XY	24	Mean	0.04	0.00	0.00	98.13	52.03	45.98	96.10	97.10
		S.D.	0.14	0.00	0.00	2.65	4.58	6.82	3.29	2.94
		C.V. (%)	—	—	—	2.7	8.8	14.8	3.4	3.0

Table 9. Summary Statistics of % Specific-signaled Nuclei by Study Site

Specimen	Lot (n)[a]	Statistics	%XY	%XX	%X	%XXX	%+21	%bi-sig 18	%bi-sig 13
100% XY	1 (8)	Mean	99.94	0.00	0.00	0.00	0.75	99.00	99.00
		S.D.	0.18	0.00	0.00	0.00	0.80	1.34	1.31
		C.V. (%)	0.2	—	—	—	—	1.4	1.3
	2 (8)	Mean	95.56	0.00	0.00	0.00	1.92	93.15	95.57
		S.D.	1.68	0.00	0.00	0.00	0.96	2.22	2.49
		C.V. (%)	1.8	—	—	—	50.0	2.4	2.6
	3 (8)	Mean	99.94	0.00	0.00	0.00	0.00	98.56	99.38
		S.D.	0.18	0.00	0.00	0.00	0.00	1.29	1.41
		C.V. (%)	0.2	—	—	—	—	1.3	1.4
10% X/ 90% XX	1 (8)	Mean	0.00	87.19	11.88	0.94	0.50	98.88	98.94
		S.D.	0.00	1.49	1.71	0.86	0.60	0.74	0.78
		C.V. (%)	—	1.7	14.4	91.5	—	0.7	0.8
	2 (8)	Mean	0.00	86.20	7.37	2.56	2.49	99.95	90.28
		S.D.	0.00	1.95	1.36	1.82	1.46	3.01	2.20
		C.V. (%)	—	2.3	18.5	71.1	58.6	3.3	2.4
	3 (8)	Mean	0.00	89.78	10.16	0.06	0.00	98.63	99.31
		S.D.	0.00	1.08	0.98	0.18	0.00	0.52	0.65
		C.V. (%)	—	1.2	9.6	—	—	0.5	0.7
17% X/ 47% XX/ 36% XXX	1 (8)	Mean	0.00	46.10	17.28	36.63	0.50	98.13	98.69
		S.D.	0.00	3.03	1.38	2.09	0.53	1.60	1.19
		C.V. (%)	—	6.6	8.0	5.7	—	1.6	1.2
	2 (8)	Mean	0.00	43.03	18.89	34.34	1.81	91.94	93.65
		S.D.	0.00	5.04	3.49	5.42	1.07	1.45	2.18
		C.V. (%)	—	11.7	18.5	15.8	59.1	1.6	2.3
	3 (8)	Mean	0.00	47.75	17.25	35.00	0.00	98.69	99.25
		S.D.	0.00	2.10	2.04	1.75	0.00	1.13	0.96
		C.V. (%)	—	4.4	11.8	5.0	—	1.1	1.0
50% XY+21/ 50% XY	1 (8)	Mean	100	0.00	0.00	0.00	48.81	99.25	98.94
		S.D.	0.00	0.00	0.00	0.00	1.07	0.46	0.82
		C.V. (%)	—	—	—	—	2.2	0.5	0.8
	2 (8)	Mean	93.01	0.00	0.44	0.00	59.08	92.94	96.08
		S.D.	2.17	0.00	0.49	0.00	6.76	1.63	1.68
		C.V. (%)	2.3	—	—	—	11.4	1.8	1.7
	3 (8)	Mean	100.00	0.00	0.00	0.00	52.81	98.88	99.94
		S.D.	0.00	0.00	0.00	0.00	3.14	1.43	0.18
		C.V. (%)	—	—	—	—	5.9	1.4	0.2

[a] (n) = No. of observations

Table 10. Summary Statistics of % Specific-signaled Nuclei by Probe-Lot

Specimen	Lot (n)[a]	Statistics	%XY	%XX	%X	%XXX	%+21	%bi-sig 18	%bi-sig 13
100% XY	6618 (6)	Mean	98.59	0.00	0.00	0.00	0.58	91.10	97.00
		S.D.	2.03	0.00	0.00	0.00	0.74	2.82	2.70
		C.V. (%)	2.1	—	—	—	—	2.9	2.8
	6619 (6)	Mean	98.33	0.00	0.00	0.00	0.83	97.08	98.75
		S.D.	2.41	0.00	0.00	0.00	1.12	3.04	2.10
		C.V. (%)	2.5	—	—	—	—	3.1	2.1
	6620 (6)	Mean	98.42	0.00	0.00	0.00	1.32	96.68	98.09
		S.D.	3.01	0.00	0.00	0.00	1.27	4.11	2.49
		C.V. (%)	3.1	—	—	—	96.2	4.3	2.5
	6621 (6)	Mean	98.58	0.00	0.00	0.00	0.83	96.76	98.09
		S.D.	2.33	0.00	0.00	0.00	1.20	3.40	2.85
		C.V. (%)	2.4	—	—	—	—	3.5	2.9
10% X/ 90% XX	6618 (6)	Mean	0.00	88.00	9.08	1.00	1.25	96.01	96.26
		S.D.	0.00	2.17	2.27	1.38	1.89	4.10	5.02
		C.V. (%)	—	2.5	25.0	—	—	4.3	5.2
	6619 (6)	Mean	0.00	87.30	10.46	1.00	1.08	95.17	96.59
		S.D.	0.00	1.83	1.76	1.14	1.42	6.24	4.90
		C.V. (%)	—	2.1	16.8	—	—	6.6	5.1
	6620 (6)	Mean	0.00	87.17	10.33	1.16	0.75	95.43	95.52
		S.D.	0.00	2.52	1.94	1.46	0.82	5.46	4.89
		C.V. (%)	—	2.9	18.8	—	—	5.7	5.1
	6621 (6)	Mean	0.00	88.42	9.33	1.59	0.91	96.67	96.35
		S.D.	0.00	2.31	3.28	2.29	1.62	3.04	4.22
		C.V. (%)	—	2.6	35.2	—	—	3.1	4.4
17% X/ 47% XX/ 36% XXX	6618 (6)	Mean	0.00	45.53	16.80	36.35	0.66	96.00	96.34
		S.D.	0.00	5.85	3.19	2.22	0.98	4.05	4.19
		C.V. (%)	—	12.8	19.0	6.1	—	4.2	4.3
	6619 (6)	Mean	0.00	44.43	17.64	37.19	1.33	96.25	97.76
		S.D.	0.00	3.74	2.62	1.38	1.33	2.95	2.91
		C.V. (%)	—	8.4	14.9	3.7	100	3.1	3.0
	6620 (6)	Mean	0.00	44.63	17.74	35.97	0.50	96.33	97.68
		S.D.	0.00	2.67	2.01	2.68	0.77	3.47	2.05
		C.V. (%)	—	6.0	11.3	7.5	—	3.6	2.1
	6621 (6)	Mean	0.00	47.92	19.05	31.78	0.58	96.42	97.01
		S.D.	0.00	2.91	2.04	4.58	0.97	3.99	2.91
		C.V. (%)	—	6.1	10.7	14.4	—	4.1	3.0
50% XY+21/ 50% XY	6618 (6)	Mean	97.83	0.00	0.17	0.00	55.28	96.76	98.18
		S.D.	3.86	0.00	0.26	0.00	6.30	3.72	1.95
		C.V. (%)	3.9	—	—	—	11.4	3.8	2.0
	6619 (6)	Mean	97.76	0.00	0.33	0.00	51.48	97.08	97.92
		S.D.	3.51	0.00	0.60	0.00	6.10	3.60	2.29
		C.V. (%)	3.6	—	—	—	11.8	3.7	2.3
	6620 (6)	Mean	97.76	0.00	0.00	0.00	54.31	97.08	98.83
		S.D.	3.56	0.00	0.00	0.00	7.98	3.61	1.75
		C.V. (%)	3.6	—	—	—	14.7	3.7	1.8
	6621 (6)	Mean	97.33	0.00	0.08	0.00	53.21	97.17	98.34
		S.D.	4.32	0.00	0.20	0.00	3.87	2.66	2.27
		C.V. (%)	4.4	—	—	—	7.3	2.7	2.3

[a] (n) = No. of observations

Table 11. Summary Statistics of % Specific-signaled Nuclei by Assay Day

Specimen	Day (n)[a]	Statistics	%XY	%XX	%X	%XXX	%+21	%bi-sig 18	%bi-sig 13
100% XY	1 (6)	Mean	98.42	0.00	0.00	0.00	1.08	96.42	97.59
		S.D.	2.22	0.00	0.00	0.00	1.27	3.10	2.91
		C.V. (%)	2.3	—	—	—	—	3.2	3.0
	2 (6)	Mean	98.42	0.00	0.00	0.00	0.91	96.59	97.59
		S.D.	3.01	0.00	0.00	0.00	1.37	3.88	2.57
		C.V. (%)	3.1	—	—	—	—	4.0	2.6
	3 (6)	Mean	98.67	0.00	0.00	0.00	1.00	97.76	98.33
		S.D.	2.08	0.00	0.00	0.00	0.77	3.28	2.89
		C.V. (%)	2.1	—	—	—	77.0	3.4	2.9
	4 (6)	Mean	98.41	0.00	0.00	0.00	0.58	96.83	98.41
		S.D.	2.46	0.00	0.00	0.00	0.97	3.01	1.94
		C.V. (%)	2.5	—	—	—	—	3.1	2.0
10% X/ 90% XX	1 (6)	Mean	0.00	87.60	9.91	1.25	1.16	94.92	96.17
		S.D.	0.00	1.86	1.54	1.21	1.35	6.05	4.59
		C.V. (%)	—	2.1	15.5	96.8	--	6.4	4.8
	2 (6)	Mean	0.00	88.33	9.33	1.67	1.16	96.92	96.26
		S.D.	0.00	1.99	2.99	2.27	1.56	3.31	4.16
		C.V. (%)	—	2.3	32.0	—	—	3.4	4.3
	3 (6)	Mean	0.00	87.50	10.17	1.00	0.50	95.26	95.77
		S.D.	0.00	3.00	2.34	1.51	0.77	5.30	5.11
		C.V. (%)	—	3.4	23.0	—	—	5.6	5.3
	4 (6)	Mean	0.00	87.45	9.80	0.83	1.17	96.17	96.51
		S.D.	0.00	2.00	2.70	1.21	1.94	4.23	5.19
		C.V. (%)	—	2.3	27.6	—	—	4.4	5.4
17% X/ 47% XX/ 36% XXX	1 (6)	Mean	0.00	45.28	17.63	35.77	0.66	96.50	95.84
		S.D.	0.00	5.65	3.06	2.84	0.98	4.36	3.89
		C.V. (%)	—	12.5	17.4	7.9	—	4.5	4.1
	2 (6)	Mean	0.00	45.88	17.29	36.09	1.00	96.42	97.43
		S.D.	0.00	4.02	2.40	1.93	1.45	2.80	2.86
		C.V. (%)	—	8.8	13.9	5.3	—	2.9	2.9
	3 (6)	Mean	0.00	45.22	19.57	33.97	0.91	95.75	97.59
		S.D.	0.00	3.17	1.96	5.72	0.97	3.64	3.08
		C.V. (%)	—	7.0	10.0	16.8	—	3.8	3.2
	4 (6)	Mean	0.00	46.13	16.74	35.47	0.50	96.33	97.93
		S.D.	0.00	3.77	2.01	2.87	0.77	3.56	2.12
		C.V. (%)	—	8.2	12.0	8.1	—	3.7	2.2
50% XY+21/ 50% XY	1 (6)	Mean	97.76	0.00	0.00	0.00	55.14	96.83	98.75
		S.D.	3.56	0.00	0.00	0.00	7.26	3.44	1.75
		C.V. (%)	3.6	—	—	—	13.2	3.6	1.8
	2 (6)	Mean	97.83	0.00	0.17	0.00	53.54	96.92	98.01
		S.D.	3.86	0.00	0.26	0.00	7.32	3.84	1.96
		C.V. (%)	3.9	—	—	—	13.7	4.0	2.0
	3 (6)	Mean	97.76	0.00	0.33	0.00	52.89	97.00	98.09
		S.D.	3.51	0.00	0.60	0.00	5.98	3.51	2.29
		C.V. (%)	3.6	—	—	—	11.3	3.6	2.3
	4 (6)	Mean	97.33	0.00	0.08	0.00	52.79	97.33	98.42
		S.D.	4.32	0.00	0.20	0.00	4.34	2.80	2.29
		C.V. (%)	4.4	—	—	—	8.2	2.9	2.3

[a] (n) = No. of observations

Table 12. Summary Statistics of % Specific-signaled Nuclei by Observer

Specimen	Observer (n)[a]	Statistics	%XY	%XX	%X	%XXX	%+21	%bi-sig 18	%bi-sig 13
100% XY	1 (12)	Mean	98.92	0.00	0.00	0.00	1.24	97.67	98.88
		S.D.	1.69	0.00	0.00	0.00	1.25	3.45	1.21
		C.V. (%)	1.7	—	—	—	—	3.5	1.2
	2 (12)	Mean	98.04	0.00	0.00	0.00	0.54	96.14	97.08
		S.D.	2.80	0.00	0.00	0.00	0.72	2.77	3.08
		C.V. (%)	2.9	—	—	—	—	2.9	3.2
10% X/ 90% XX	1 (12)	Mean	0.00	88.21	10.40	1.42	1.45	96.50	96.80
		S.D.	0.00	1.84	2.47	1.31	1.76	3.88	3.74
		C.V. (%)	—	2.1	23.8	92.3	—	4.0	3.9
	2 (12)	Mean	0.00	87.23	9.21	0.96	0.54	95.13	95.56
		S.D.	0.00	2.37	2.06	1.76	0.75	5.27	5.18
		C.V. (%)	—	2.7	22.4	—	—	5.5	5.4
17% X/ 47% XX/ 36% XXX	1 (12)	Mean	0.00	46.66	18.48	36.30	1.04	96.71	97.85
		S.D.	0.00	3.307	2.12	2.52	1.28	3.53	2.81
		C.V. (%)	—	7.1	11.50	6.90	—	3.7	2.90
	2 (12)	Mean	0.00	44.60	17.13	34.34	0.50	95.79	96.55
		S.D.	0.00	4.47	2.73	4.12	0.60	3.35	3.08
		C.V. (%)	—	10.0	15.9	12.0	—	3.5	3.2
50% XY+21/ 50% XY	1 (12)	Mean	98.25	0.00	0.21	0.00	54.56	97.84	98.80
		S.D.	2.65	0.00	0.45	0.00	5.74	2.96	1.50
		C.V. (%)	2.7	—	—	—	10.50	3.0	1.50
	2 (8)	Mean	97.09	0.00	0.08	0.00	52.58	96.21	97.83
		S.D.	4.35	0.00	0.19	0.00	6.32	3.34	2.31
		C.V. (%)	4.5	—	—	—	12.0	3.5	2.4

[a] (n) = No. of observations

Methods Comparison; Clinical Specimens

A multi-center, blinded, controlled, comparative study was conducted to further define the performance characteristics of the AneuVysion kit relative to standard chromosome analysis, the standard of care, in cultured and uncultured amniotic fluid specimens. Thirty one investigation sites analyzed amniotic fluid specimens obtained from a total of 1,516 patients with one or more of the risk factors listed below.

1. Advanced maternal age: 978 specimens
2. Abnormal ultrasound: 751 specimens
3. Abnormal maternal screening profile: 591 specimens
4. Family history: 78 specimens
5. Elevated maternal serum Alpha-Fetoprotein: 72 specimens
6. Maternal anxiety: 15 specimens
7. Other: 75 specimens

All study sites conducted the trial according to the prescribed assay procedures and signal enumeration guides.

A total of 2,238 amniocyte specimens were obtained and analyzed from 1,516 patients. Of these 2,238 specimens, 55 were deemed uninformative. These 55 uninformative specimens included three due to maternal cell contamination, four due to FISH assay failures and 48 had an insufficient (< 40) number of nuclei for analysis. Thus on a per specimen basis, the rate of informativeness is 97.5% (2183/2238). Note that among the 48 uninformative cases due to an insufficient (< 40) number of nuclei available for 1 or both of the probes, 31 were partially uninformative for either the CEP 18/X/Y (22) or the LSI 13/21 (9).

Of the 1,516 patients, 13 patients with either cultured or uncultured specimens were included in the 55 uninformative specimens. Thus, on a per patient basis, the rate of informativeness is 99.8% (1503/1516). One specimen per patient was included in the primary analyses. Of these, 589 were cultured and 927 were uncultured specimens. **Table 13** summarizes the study specimens by karyotype and specimen type.

Table 13. Summary of Specimen Enrollment by Aneuploidy

Aneuploidy	Cultured	Direct	Total
Normal	76	435	511
+13	34	41	75
+18	107	87	194
+21	156	167	323
-21	0	1	1
triploid	12	21	33
tetraploid	1	0	1
X	54	54	108
XXX	31	15	46
XXY	45	12	57
XXXXY	1	0	1
XXY,+18	1	0	1
XXX,+18	0	1	1
XXYY	1	0	1
XYY	17	7	24
45,XX,idic(18)	0	1	1
pseudomosaic	14	62	76
mosaic	39	23	62
Total	**589**	**927**	**1516**

The maternal age ranged from 13 to 52 years, with a mean (±S.D.) age of 33.2 years (±6.8 years). The gestational age ranged from 11 to 38 weeks, with a mean (±S.D.) of 18.8 weeks (±4.6 weeks). The mean maternal age varied among study sites, while the mean gestational age did not.

Comparison of Test Results by FISH and Standard Chromosome Analysis

Each site performed FISH analyses according to the instructions in the AneuVysion kit package insert. The percentage of aneuploid cells was determined by FISH after enumerating a minimum of 50 interphase nuclei per target; a minimum of 40 evaluable nuclei was deemed informative.

From this pivotal multi-center comparative study described above, the results of interphase FISH analysis were compared to standard chromosome analysis, which is shown in **Table 14**.

Table 14. % Aneuploid Cells Obtained by FISH Assay, by Categories of Standard Chromosome Analysis. All Sites Combined and All Specimens Informative for Both Probes

	% Aneuploid cells	Standard Cytogenetic Chromosome Analysis				
		Aneuploidy[a]	Mosaic[b]	Pseudomosaic[c]	Euploidy	Total
F	> 60%	860[d]	15[e]			875
I	10-60%	1[f]	35			36
S	< 10%		12[g]	76	504[h]	592
H	failure	6			7	13
	Total	**867**	**62**	**76**	**511**	**1516**

[a] Includes 75 +13; 192 +18; 322 +21; 107 45,X; 44 47,XXX; 57 47,XXY, 24 47,XYY; one -21; one XXY +18; one XXX +18; one 49,XXXXY; one 48,XXYY; one tetraploid 92,XXYY, and 33 triploids (19 69,XXX and 14 69,XXY).

[b] Includes 23 cases associated with 45,X, 10 with XXX, 8 with XXY, 7 with +21, 4 with +18, 2 with -18, 3 with +13, and 5 with 45,X complexes. Although aneuploid cell lines were detected in 60 cases, the % aneuploid cell varied.

[c] Includes 7 +13, 9 -13, one +18, 24 -18, 11 +21, 19 -21, 25 X0, one XXX, one XXY, one XYY, and 6 XXYY.

[d] Includes one case of 47,XX,+21 and one case of 47,XY,+21 with mild maternal cell contamination.

[e] In 8 of these 15 cases, standard cytogenetic analysis also showed the % aneuploid metaphases to be ≥ 60%.

[f] One case with 46,XX,idic(18), FISH result was 35% +18 and 96% XX in uncultured specimen. FISH assay of the cultured sample of the same specimen showed 100% for both +18 and XX.

[g] In 7 of these 12 cases, standard cytogenetic analysis also showed the % aneuploid metaphases to be ≤ 10%.

[h] Includes one case of 47,XY,+22 and three cases of 46,XY with mild maternal cell contamination.

True Aneuploid Cases

Among the 861 aneuploid cases, there were 75 + 13; 192 + 18; 322 + 21; 107 45,X; 44 47,XXX; 57 47,XXY, 24 47,XYY; one -21; one XXY +18; one XXX + 18; one 49,XXXXY; one 48,XXYY; one tetraploid 92,XXYY, and 33 triploids (19 69,XXX and 14 69,XXY) and one 46,XX,idic(18). Of which, 860 had % aneuploid cells by FISH greater than 60% and one 35% was due to long storage of the prepared slide. Thus, under the worst case scenario, for determination of true aneuploidy, FISH is able to detect 99.9% (860/861) of cases identified by standard chromosome analysis. Note also that one male and one female trisomy 21 fetal cases with mild maternal cell contamination were deemed informative.

Mosaic Cases

There were 62 true mosaic cases, as identified by standard chromosome analysis. There were 23 cases associated with 45,X, 10 with XXX, 8 with XXY, 7 with +21, 4 with +18, 2 with -18, 3 with +13, and 5 with X0 complexes. Even though aneuploid cell lines were detected in 60 cases, twelve of the 62 mosaic cases showed less than 10% aneuploid cells by FISH, and 15 showed greater than 60%. The correlation of the % aneuploid cells is 0.76 between FISH and standard chromosome analysis.

Pseudomosaic Cases

There were 76 pseudomosaic cases, as identified by standard chromosome analysis. Among these 76 cases, 7 +13, 9 -13, one +18, 24 -18, 11 +21, 19 -21, 25 X0, one XXX, one XXY, one XYY, and 6 XXYY were observed. FISH assay results showed less than 10% aneuploid cells, which is consistent with the euploid state.

Euploid Cases

Among 504 euploid cases identified by standard chromosome analysis, FISH also found each to have % aneuploid cells less than 10%. Thus, for determination of euploidy, FISH is able to detect 100% (504/504) of cases identified by standard chromosome analysis. Note also that all four male fetal cases with mild maternal cell contamination were deemed informative.

Pairwise Comparison between Cultured and Uncultured Specimens

There were 722 patients in the pivotal study with FISH assay performed on both uncultured and cultured samples of the same specimen. The pairwise comparison of FISH assays on these 722 uncultured - cultured paired samples are shown in **Table 15**. In mosaic cases, the aneuploid cell lines were detected by FISH with varying % aneuploid cells between cultured and uncultured samples, leading to a few discordants. The FISH test results in aneuploid, euploid and pseudomosaic cases were concordant between cultured and uncultured samples.

Table 15. Comparison of FISH Assay Results on Uncultured vs. Cultured Samples of the Same Specimen

	Uncultured					
Cultured	**Aneuploidy[a]**	**Mosaic[b]**	**Pseudo-mosaic**	**Euploidy**	**Failure[c]**	**Total**
Aneuploid	299	2[d]			19	**320**
Mosaic	1[d]	9		1[d]		**11**
Pseudo-mosaic			54		9	**63**
Euploid		1[d]		313	11	**325**
Failure[c]		1	1	1		**3**
Total	**300**	**13**	**55**	**315**	**39**	**722**

[a] Includes 27 +13; 66 +18; 147 +21; 25 45,X; 10 47,XXX; 11 47,XXY; 4 47,XYY; 9 triploid; one 46,XX,idic(18); one 46,XX,-21,+mar, der(21)(wcp21+)

[b] Includes 5 45,X; 2,XXY; one XXX; one +13; one +18; one +21; and one -18. Even though all aneuploid cell lines were detected, the % aneuploid cell varied.

[c] Forty-two patients had both uncultured and cultured sample pairs of which 19 were aneuploidy, 10 pseudomosaic, 12 euploid, and 1 mosaic.

[d] Although the same aneuploid and/or euploid cell lines were detected by FISH assay, the % aneuploid cells varied between cultured and uncultured samples.

BIBLIOGRAPHY

1. American College of Medical Genetics. Technical and clinical assessment of fluorescence in situ hybridization: an ACMG/ASHG position statement. I. Technical considerations. *Genet Med.* 2000;2(6):356-361.
2. Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. *Br Med J*. 1988;297:883-887.
3. Canick JA, Palomaki GE, Osthanondh R. Prenatal screening for trisomy 18 in the second trimester. *Prenat Diagn*. 1990;10:546-548.
4. Ried T, Landes G, Dackowski W, et al. Multicolor fluorescence in situ hybridization for the simultaneous detection of probe sets for chromosomes 13, 18, 21, X and Y in uncultured amniotic fluid cells. *Hum Mol Genet.* 1992;1:307-313.
5. Ward BE, Gersen SL, Carelli MP, et al. Rapid prenatal diagnosis of chromosomal aneuploidies by fluorescence in situ hybridization: clinical experience with 4,500 specimens. *Am J Hum Genet.* 1993;52:854-865.
6. Robinson A, Puck M, Pennington B, et al. Abnormalities of the sex chromosomes: a prospective study on randomly identified newborns. *Birth Defects.* 1979;15:203-241.
7. West JD, Gosden CM, Gosden JR, et al. Sexing the human fetus and identification of polyploid nuclei by DNA-DNA in situ hybridization in interphase nuclei. *Mol Reprod Dev.* 1989;1:129-137.
8. Cacheux V, Tachdjian G, Druart L, et al. Evaluation of X, Y, 18, and 13/21 alpha satellite DNA probes for interphase cytogenetic analysis of uncultured amniocytes by fluorescence in situ hybridization. *Prenat Diagn.* 1994;14:79-86.
9. Romana SP, Tachdjian G, Druart L, et al. A simple method for prenatal diagnosis of trisomy 21 on uncultured amniocytes. *Eur J Hum Genet.* 1993;1:245-251.
10. Zheng YL, Ferguson-Smith MA, Warner JP, et al. Analysis of chromosome 21 copy number in uncultured amniocytes by fluorescence in situ hybridization using a cosmid contig. *Prenat Diagn.* 1992;12:931-943.
11. Lebo RV, Flandermeyer RR, Diukman R, et al. Prenatal diagnosis with repetitive in situ hybridization probes. *Am J Med Genet.* 1992; 43:848-854.
12. Zahed L, Murer-Orlando M, Vekemans M. In situ hybridizations studies for the detection of common aneuploidies in CVS. *Prenat Diagn* 1992;12:483-493.
13. Christensen B, Bryndorf T, Philip J, et al. Rapid prenatal diagnosis of trisomy 18 and triploidy in interphase nuclei of uncultured amniocytes by non-radioactive in situ hybridization. *Prenat Diagn.* 1992;12:241-250.
14. Gersen SL, Carelli MP, Ward BE. A retrospective statistical study of 4,500 prenatal FISH analyses: trisomic vs. disomic cutoff thresholds for autosomal aneuploidies. *Am J Hum Genet Suppl.* 1993; 53:A1411.
15. Ward BE, Wright M, Lytle C, et al. Prenatal aneuploidy detection by fluorescence in situ hybridization (FISH) in 1,068 second trimester pregnancies with fetal ultrasound abnormalities. *Am J Hum Genet Suppl.* 1994;55:A1696.
16. Kuo W-L, Tenjin H, Segraves R, et al. Detection of aneuploidy involving chromosomes 13, 18, or 21 by fluorescence in situ hybridization (FISH) to interphase and metaphase amniocytes. *Am J Hum Genet.* 1991;49:112-119.
17. Tepperberg J, Pettenati MJ, Rao PN, et al. Prenatal diagnosis using interphase fluorescence in situ hybridization (FISH): two-year multi-center retrospective study and review of the literature. *Prenat Diagn.* 2001;21(4):293-301.
18. U.S. Centers for Disease Control. *Morbidity and Mortality Weekly Review.* 1987;36 (suppl. 2S):2S-18S.
19. Barch MJ, ed. The ACT Cytogenetics Laboratory Manual. 2nd ed. New York, NY: *Raven Press, Ltd;* 1991:17-105, 297-536.
20. Winsor EJT, Silver MP, Theve R, et al. Maternal cell contamination in uncultured amniotic fluid. *Prenat Diagn.* 1996;16:49-54.
21. Hsu LYF, Kaffe S, Jenkins EC, et al. Proposed guidelines for diagnosis of chromosome mosaicism in amniocytes based on data derived from chromosome mosaicism and pseudomosaicism studies. *Prenat Diagn.* 1992;12:555-573.
22. ACOG Practice Bulletin No. 88: Invasive Prenatal Testing for Aneuploidy. *Obstet Gynecol.* 2007;110(6):1459-67.
23. Cunniff C, et al. Prenatal screening and diagnosis for pediatricians. *Pediatrics.* 2004;114(3):889-94.

24. Hong Kong College of Obstetricians and Gynaecologists. Guidelines for genetic amniocentesis. *HKCOG Guidelines* [serial online]. July 2000;4:1-4. Available at: http://www.hkcog.org.hk/docs/guidelines/Genetic%20Amniocentesis_2000.pdf. Accessed April 19, 2010.
25. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*, Fifth Edition. Washington, DC: US Government Printing Office, December 2009.
26. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
27. 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.
28. World Health Organization. *Laboratory Biosafety Manual.* 3rd ed. Geneva, Switzerland: World Health Organization; 2004.

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Abbott Molecular Inc.
1300 East Touhy Avenue
Des Plaines, IL 60018 USA

CE 0843

EC REP Abbott GmbH & Co. KG
Max-Planck-Ring 2
65205 Wiesbaden
Germany

www.abbottmolecular.com

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