Publications

Kilpatrick MW et al. Am J Obstet Gynecol 2004;190:1571

Automated detection of rare fetal cells in maternal blood: eliminating the false-positive XY signals in XX pregnancies

OBJECTIVE: The purpose of this study was to develop a new method to help differentiate XX from XY signals in maternal blood from women carrying XY fetuses.

STUDY DESIGN: We have developed a system to scan automatically for cells that bear X and Y fluorescence in situ hybridization signals. These XY target cells are identified by scans at low (x20) magnification, and all identified targets are revisited and verified at high (x100) magnification. The viewer software component of the system displays x20 images of all cells and intracellular fluorescence in situ hybridization signals that are present in each of the 4000 optical fields per slide, along with x100 images of automatically detected target cells.

RESULTS: We initially examined 36,000 fields from 18 slides in 12 pregnancies (6 male and 6 female) using our system that is based on fluorescence in situ hybridization with a single probe for the X-chromosome and a single probe for the Y-chromosome and found XY nuclei in all samples, regardless of fetal gender. In the second phase of the study, a refinement of the approach that incorporated 2 independent probes for the Y-chromosome resulted in a false-positive rate for detection of XY nuclei in XX cases <0.00005%.

CONCLUSION: Our data suggest that this system may allow for excellent "signal to noise" separation, which is required absolutely for fetal cell methods to differentiate aneuploid from normal pregnancies. Quantitation of fetal cells in the maternal circulation and standardization of processes that have been developed for their enrichment are crucial to moving fetal cell assessment from esoteric basic science to applied new technology.

Evans et al. FetalDiagnTher 2006;21:523

Automated microscopy of amniotic fluid cells: detection of FISH signals using the FastFISH imaging h

OBJECTIVE: FISH (fluorescence in situ hybridization) analysis is a valuable adjunct to cytogenetics that provides a rapid screen for common abnormalities. However, FISH is expensive, labor-intensive, and requires a high skill level and subjective signal interpretation. A fully automated system for FISH analysis could improve laboratory efficiency and potentially reduce errors and costs.

METHODS: In this study we blindly compared automated FISH signal acquisition and display against standard FISH analysis. A total of 62 amniocentesis samples were prepared using the AneuVysion multicolor DNA probe kit and probed for chromosomes 13, 18, 21, X, and Y. Two sets of slides were produced from each sample. Fifty cells were scored in each slide. One set was evaluated using standard manual microscopy and the other using the automated image acquisition and display capabilities of the Ikoniscope fastFISH amnio Test System. This system uses epifluorescence optics, along with optimized slide management to process slides automatically.

RESULTS: A 100% concordance was observed between the results obtained using manual microscopy and the automated system. There was also 100% concordance between the FISH results and those obtained by conventional karyotyping.

CONCLUSION: Our data suggest that the automated system is capable of providing accurate and rapid identification and display of cells and FISH signals.

Wauters PrenatDiagn 2007;27:951

Fully automated FISH examination of amniotic fluid cells.

OBJECTIVE: Fluorescence in situ hybridization (FISH) analysis has become a valuable adjunct in cytogenetics, providing a rapid screen for common chromosome abnormalities that is particularly helpful in prenatal diagnosis. FISH analysis using standard microscopy is expensive and labor intensive, requiring both a high skill level and subjective signal interpretation. A reliable fully automated system for FISH analysis could improve laboratory efficiency and potentially reduce errors and costs.

METHODS: The efficacy of an automated system was compared to standard manual FISH analysis. Two sets of slides were generated from each of 152 amniotic fluid samples. Following hybridization with a standard panel of five chromosome FISH probes, one set of slides was evaluated using manual microscopy. The other set was evaluated using an automated microscopy system.

RESULTS: A diagnostic outcome was obtained for all 152 samples using manual microscopy and for 146 of 152 (96%) samples using automated microscopy. Three cases of aneuploidy were detected. For those samples for which a diagnostic outcome was determined by both manual and automated microscopy, 100% concordance was observed. All FISH analysis results were confirmed by karyotype.

CONCLUSION: These data suggest that an automated microscopy system is capable of providing accurate and rapid enumeration of FISH signals in amniocytes.

Yu C ICPR 2008

Her-2/neu Status Detection in Fluorescence In-Situ Hybridization (FISH) Image Stack

To determine the Her-2/neu status in breast specimen slides, accurate automated dot counting is highly
desirable. Existing methods for dot counting in Fluorescence in situ hybridization (FISH) images are using
thresholding on original intensity images. In this paper, we present a gradient-based dot detection method. FISH dot segmentation procedure consists of a tophat preprocessing and gradient calculation for each plane in the image stack. Adaptive thresholds are found based on maximum gradient images. Pixels with gradient above threshold grow into dots. Previously suggested size, shape and intensity features are extended to 3D case. The intensity features are normalized by surrounding background intensity to characterize local contrast. Real FISH signals are recognized by trained multi-layer perceptron (MLP). The proposed method was verified with several breast cancer slides.

Ntouropi TG et al. BrJCancer 2008;99:789

Detection of circulating tumour cells in peripheral blood with an automated scanning fluorescence microscope.

We have developed an automated, highly sensitive and specific method for identifying and enumerating circulating tumour cells (CTCs) in the blood. Blood samples from 10 prostate, 25 colorectal and 4 ovarian cancer patients were analysed. Eleven healthy donors and seven men with elevated serum prostate-specific antigen (PSA) levels but no evidence of malignancy served as controls. Spiking experiments with cancer cell lines were performed to estimate recovery yield. Isolation was performed either by density gradient centrifugation or by filtration, and the CTCs were labelled with monoclonal antibodies against cytokeratins 7/8 and either AUA1 (against EpCam) or anti-PSA. The slides were analysed with the Ikoniscope

robotic fluorescence microscope imaging system. Spiking experiments showed that less than one epithelial cell per millilitre of blood could be detected, and that fluorescence in situ hybridisation (FISH) could identify chromosomal abnormalities in these cells. No positive cells were detected in the 11 healthy control samples. Circulating tumour cells were detected in 23 out of 25 colorectal, 10 out of 10 prostate and 4 out of 4 ovarian cancer patients. Five samples (three colorectal and two ovarian) were analysed by FISH for chromosomes 7 and 8 combined and all had significantly more than four dots per cell. We have demonstrated an Ikoniscope based relatively simple and rapid procedure for the clear-cut identification of CTCs. The method has considerable promise for screening, early detection of recurrence and evaluation of treatment response for a wide variety of carcinomas.

Seppo A et al. PrenatDiagn 2008;28:815

Detection of circulating fetal cells utilizing automated microscopy: potential for noninvasive prenatal diagnosis of chromosomal aneuploidies.

OBJECTIVE: As fetal cells can be indisputably identified through detection of Y FISH signals, we utilized an automated microscopy system developed to identify and enumerate cells bearing X and Y FISH signals. We further investigated the potential of fetal hemoglobin expression as a gender independent marker for automated identification of fetal cells.
METHOD: For FISH-based scanning, verified fetal cells were identified based on the presence of a single X-signal and individual signals for each of the two Y FISH probes. For cell identification based on fetal hemoglobin expression, putative fetal cells were verified based on the presence of signals for anti-gamma or anti-epsilon globin antibody, and FISH signals for the X- and Y- chromosomes.
RESULTS: Fetal cells were identified, by FISH-based scanning, in 28 of the 29 maternal samples from pregnancies with male fetuses. Simple density gradient centrifugation achieved a 3- to 5-fold increase in the number of fetal cells detected.
CONCLUSION: Automated microscopy identified fetal cells in both first and second trimester maternal blood samples. Although we were unable to detect fetal erythroblasts in numbers sufficient for clinical diagnosis, the ability to reliably detect fetal cells by FISH-based scanning opens the possibility for prenatal detection of chromosomal aberrations utilizing circulating fetal cells.

Seppo A et al. GynecolOncol 2009;114:80

Gain of 3q26: a genetic marker in low-grade squamous intraepithelial lesions (LSIL) of the uterine cervix.

OBJECTIVE: Physicians have few resources for determining which LSIL will progress to HSIL or regress. Recently the chromosome 3q26 region was found to be amplified in patients with cervical cancer. The frequency of this 3q gain increased with severity of dysplasia. The primary objective of this study was to evaluate an automated FISH assay for detection of 3q gain in liquid cytology samples as a potential tool for risk stratification and triaging.
METHODS: Slides prepared from 257 liquid cytology specimens (97 Negative, 135 LSIL 25 HSIL) were hybridized with a single-copy probe for the chromosome 3q26 region and a probe for the centromeric alpha-repeat sequence of chromosome 7, using standard FISH methods. Using automated analysis, the total number of nuclei and the number of nuclei with >2 signals for 3q26 were determined, using a 20x objective. The nuclei were rank ordered based on number

of 3q26 FISH signals. The 800 nuclei with the highest number of signals were scored using both FISH probes and nuclei with increased numbers of 3q signals were enumerated.
RESULTS AND CONCLUSIONS: Analysis of 257 specimens demonstrated that a fully automated FISH scoring system can detect 3q gain in liquid cytology samples. A fully automated method for determination of 3q gain in liquid cytology may be the assay necessary to implement routine testing. Additional studies to validate the utility of this technology are needed.

Ashraf SQ BrJCancer 2009;101:1758
Humanised IgG1 antibody variants targeting membrane-bound carcinoembryonic antigen by antibody-dependent cellular cytotoxicity and phagocytosis.
BACKGROUND: The effect of glycoengineering a membrane specific anti-carcinoembryonic antigen (CEA) (this paper uses the original term CEA for the formally designated CEACAM5) antibody (PR1A3) on its ability to enhance killing of colorectal cancer (CRC) cell lines by human immune effector cells was assessed. In vivo efficacy of the antibody was also tested.
METHODS: The antibody was modified using EBNA cells cotransfected with beta-1,4-N-acetylglucosaminyltransferase III and the humanised hPR1A3 antibody genes.
RESULTS: The resulting alteration of the Fc segment glycosylation pattern enhances the antibody's binding affinity to the FcgammaRIIIa receptor on human immune effector cells but does not alter the antibody's binding capacity. Antibody-dependent cellular cytotoxicity (ADCC) is inhibited in the presence of anti-FcgammaRIII blocking antibodies. This glycovariant of hPR1A3 enhances ADCC 10-fold relative to the parent unmodified antibody using either unfractionated peripheral blood mononuclear or natural killer (NK) cells and CEA-positive CRC cells as targets. NK cells are far more potent in eliciting ADCC than either freshly isolated monocytes or granulocytes. Flow cytometry and automated fluorescent microscopy have been used to show that both versions of hPR1A3 can induce antibody-dependent cellular phagocytosis (ADCP) by monocyte-derived macrophages. However, the glycovariant antibody did not mediate enhanced ADCP. This may be explained by the relatively low expression of FcgammaRIIIa on cultured macrophages. In vivo studies show the efficacy of glycoengineered humanised IgG1 PR1A3 in significantly improving survival in a CRC metastatic murine model.
CONCLUSION: The greatly enhanced in vitro ADCC activity of the glycoengineered version of hPR1A3 is likely to be clinically beneficial.

Jalali GR et al. AJOG AmJObstetGynecol 2010;202:581
Amplification of the chromosome 3q26 region shows high negative predictive value for nonmalignant transformation of LSIL cytologic finding.
OBJECTIVE: The chromosome 3q26 region is a biomarker for cervical cancer. Women with low-grade squamous intraepithelial lesions (LSIL) currently are referred for immediate colposcopy. The objective of this study was to determine the negative predictive value of the 3q26 amplification test for the persistence or regression of LSIL.
STUDY DESIGN: Archival thin layer cytologic slides of 47 women (14-67 years old) with LSIL were linked to histologic and cytologic end points. To determine 3q status, the slides were hybridized for the chromosome 3q26 region and for the centromere of chromosome 7, as a control, with the use of the standard fluorescent in situ hybridization methods.
*RESULTS:*The negative predictive value of 3q26 gain for the development of cervical intraepithelial neoplasia grade 2/3 within 1 year was 93% (95% confidence interval, 68- 100); after 21 months, its negative predictive value was 100% (95% confidence interval, 29-100).

CONCLUSION: The 3q26 gain might help identify women with LSIL who do not need colposcopy.

Sifakis S et al. EarlyHumanDev 2010;86:311

Prenatal diagnosis of trisomy 21 through detection of trophoblasts in cervical smears.

BACKGROUND: Fetal cells exfoliate in the uterine cavity during early pregnancy and are a potential source of material for NIPD.

AIMS: This study was designed to test the hypothesis that fetal cells obtained from the uterine cervix during the first trimester of pregnancy could be utilized for prenatal diagnosis of chromosomal aneuploidy.

STUDY DESIGN: Fetal cells retrieved from the distal endocervical canal during the first trimester of pregnancy were hybridized with chromosome 21 specific FISH probes and analyzed with an automated fluorescence microscope.

SUBJECTS AND OUTCOME MEASURES: Cells with 3 copies of chromosome 21 were detected in 5 out of 5 trisomy 21 pregnancies.

RESULTS: The number of trisomic cells detected ranged from 1 to 27 with a median value of 5.

CONCLUSIONS: FISH-based scanning can identify trisomy 21 pregnancies by analysis of routine cervical brushings. The approach offers the potential for non-invasive prenatal diagnosis as early as 5 weeks gestation.

Verri A LabMedicine 2011;42:134

Significant Progression of Uterine Cervical Epithelial Lesion Accompanied by Marked Increase in 3q26 Gene Amplification

Studies have demonstrated a correlation between the gain in 3q26 copy number and the severity and stage of cervical disease progression.7,9,10 A recent study has examined the potential of using a measure of 3q26 gain as a predictor of regression, persistence, or progression of low-grade squamous intraepithelial lesions (LSIL) of the cervix.12 The case described in this report documents a marked progression in both the severity and extent of this patient's lesion from atypical squamous cells on cytology to biopsy established CIN2-CIN3 over the span of 1 year. The initial gain of at least 5 copies of 3q26 in only 3 nuclei in this patient's first cervical smear may be an indication of the significance and sensitivity of this degree of gain, even in a small number of cells at a low level of disease, and may suggest the potential of predicting the progression of the lesion. The subsequent gain of at least 5 copies of 3q26 1 year later in the very large number of 264 nuclei may reflect both the severity and extent of disease progression. Nevertheless, since it is not possible to exclude the possibility that a high-grade CIN already existed at the time of the initial cytology, the presence of the 3q26 gain, even in a small number of cells, may also serve as an indicator of the possible presence of a high-grade lesion in those cervical specimens in which a definitive cytological diagnosis is not or cannot be made. This case report supports the findings of other investigators on the potential utility of using 3q26 gain in predicting, at an early stage, the progression or non-progression of low-grade preneoplastic lesions of the cervix.

Kilpatrick MW et al. Anal Quant Cytol Histol 2011;33:205

Determination of HER2 gene status by fully automated fluorescence microscopy.

OBJECTIVE: To examine fluorescence in situ hybridization (FISH) in HER2 amplification in response rates to trastuzumab therapy and both taxane and anthracycline-based chemotherapy regimens.
STUDY DESIGN: A total of 400 tumor sections were analyzed over an 8-month period. The sections were hybridized with probes for the HER2 gene and chromosome 17 centromere using standard FISH methods and analyzed on an automated fluorescence microscopy system.
RESULTS:Reliable and valid methods for identification of the patients that will respond to treatment with trastuzumab are needed in order to achieve maximum therapy efficacy and maintain cost efficiency. FISH-based analysis is potentially an objective and reproducible approach to determination of HER2 gene status; however, manual FISH counting is a laborious task and subject to inter and intraobserver variability.
CONCLUSION: The system described in this paper is a valuable tool in providing a consistent approach to the interpretation of breast tumor tissue analyzed by FISH analysis. In addition to consistency, an automated system provides a record of the images produced that can be of immediate benefit in multiple review of a difficult or equivocal case and long-term benefit in terms of providing a permanent case record.

Marganski WA et al. CancerCytopathol 2011;119:279-89
Digitized microscopy in the diagnosis of bladder cancer: analysis of >3000 cases during a 7-month period.
BACKGROUND: Fluorescent in situ hybridization (FISH) analysis of urine samples has proven to be a valuable adjunctive test to urine cytology for both diagnosis and monitoring recurrence of urothelial carcinoma. Automated FISH analysis has the potential to improve laboratory efficiency and to reduce interobserver and intraobserver variability, resulting in more accurate, reproducible, assay performance.
METHODS: A total of 3200 slides containing urine specimens, hybridized with the UroVysion Bladder Cancer Kit (Abbott Molecular, Des Plaines, Illinois), a 4-probe set for chromosomes 3, 7, 17, and 9p21, was evaluated at Acupath Laboratories. The slides were analyzed over a 7-month period, using the Ikoniscope - oncoFISH bladder Test System (Ikonisys, New Haven, Connecticut).
RESULTS: Analysis included the incorporation of a "flagging" system developed by Acupath Laboratories to identify cases, based on specific criteria, likely to benefit from further manual review. By using US Food and Drug Administration (FDA)-cleared scoring criteria, 96.3% of the slides could be reported directly from the automated scan, requiring no manual review of the slide. For the remaining 3.7% of the samples (all of which were very hypocellular), a manual review of each slide subsequently allowed diagnoses to be successfully reported. The average scan time was 31.7 minutes, and the average slide scan review time was 8.3 minutes.
CONCLUSIONS: This study demonstrated the value of an automated approach to the analysis of FISH slides, affording the benefit of high-throughput while providing the user with the necessary images and tools to quickly and accurately report a case.

Rodolakis A et al. IntJGynecolCancer 2012;22):742
Role of chromosome 3q26 gain in predicting progression of cervical dysplasia.
OBJECTIVE: This study aimed to determine whether 3q26 gain can predict which low-grade squamous intraepithelial lesions (LSILs) and atypical squamous cells of undetermined significance (ASCUSs) will progress to higher-grade squamous intraepithelial lesion (HSIL).

METHODS: Liquid cytology specimens of LSIL and ASCUS from 73 women were examined using fluorescent in situ hybridization (FISH) for the detection of 3q26 gain. All women underwent colposcopy and biopsy at the initial visit and 40 of them with histology showing cervical intraepithelial neoplasia 1 (CIN 1) or human papillomavirus infection (koilocytosis) were included in the study. They were reevaluated with liquid cytology, colposcopy, and biopsy after a median follow-up of 17.5 months.
RESULTS: A total of 40 cases were analyzed (31 LSILs and 9 ASCUSs). Of these cases, 8 (20%; 6 LSILs and 2 ASCUSs) were positive and 32 (80%) were negative for 3q26 gain according to FISH. Three of the 8 positive women (38%) progressed to HSIL/CIN 2 or worse, whereas none of the 32 negative women did so. 3q26 gain could predict progression with a negative predictive value of 100% (95% confidence interval, 89.1%-100%). In addition, women positive for 3q26 gain had a significantly lower regression rate compared with negative women (P = 0.009).
CONCLUSIONS: In this first prospective study, 3q26 gain in LSIL/ASCUS cytology exhibited an impressive negative predictive value for progression to HSIL/CIN 2 or worse. Thus, 3q26 gain may be useful in stratifying patients' risk for progression and possibly alter management and reduce cost of follow-up.

Heitmann et al. PLoS ONE 2012;7:e39101
3q26 amplification is an effective negative triage test for LSIL: a historical prospective study.
Background: Women with low grade squamous intraepithelial lesions (LSIL) at cervical cancer screening are currently referred for further diagnostic work up despite 80% having no precancerous lesion. The primary purpose of this study is to measure the test characteristics of 3q26 chromosome gain (3q26 gain) as a host marker of carcinogenesis in women with LSIL. A negative triage test may allow these women to be followed by cytology alone without immediate referral to colposcopy.
Methods and Findings: A historical prospective study was designed to measure 3q26 gain from the archived liquid cytology specimens diagnosed as LSIL among women attending colposcopy between 2007 and 2009. 3q26 gain was assessed on the index liquid sample; and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were measured at immediate triage and at 6–16 months after colposcopic biopsy. The sensitivity of 3q26 gain measured at immediate triage from automated and manually reviewed tests in 65 non-pregnant unique women was 70% (95% CI: 35, 93) with a NPV of 89% (95% CI: 78, 96). The sensitivity and NPV increased to 80% (95% CI: 28, 99) and 98% (95% CI: 87, 100), respectively, when only the automated method of detecting 3q26 gain was used.
Conclusions: 3q26 gain demonstrates high sensitivity and NPV as a negative triage test for women with LSIL, allowing possible guideline changes to routine surveillance instead of immediate colposcopy. Prospective studies are ongoing to establish the sensitivity, specificity, PPV and NPV of 3q26 gain for LSIL over time.

Emad A et al. BiomedBiotech 2012;2012:610856
Efficiency of manual scanning in recovering rare cellular events identified by fluorescence in situ hybridization: simulation of the detection of fetal cells in maternal blood.
Fluorescence in situ hybridization (FISH) and manual scanning is a widely used strategy for retrieving rare cellular events such as fetal cells in maternal blood. In order to determine the efficiency of these techniques in detection of rare cells, slides of XX cells with predefined numbers (1-10) of XY cells were prepared. Following FISH hybridization, the slides were

scanned blindly for the presence of XY cells by different observers. The average detection efficiency was 84% (125/148). Evaluation of probe hybridization in the missed events showed that 9% (2/23) were not hybridized, 17% (4/23) were poorly hybridized, while the hybridization was adequate for the remaining 74% (17/23). In conclusion, manual scanning is a relatively efficient method to recover rare cellular events, but about 16% of the events are missed; therefore, the number of fetal cells per unit volume of maternal blood has probably been underestimated when using manual scanning.

***Romano P et al*. LabMedicine *2013;44:267*3**

3q26 Gene Amplification in a Woman With Abnormal Cervical Cytology Unconfirmed by Cervical Biopsies

The patient had abnormal cervical cytology (Pap test) assessments but non-diagnostic biopsies, posing a
treatment dilemma for the clinician. Would the lesion regress as purported to occur in many of these lesions? If not, there would be the risk of under-treatment. If it were to regress, there would be unnecessary over-treatment, with the potential for subsequent obstetric problems during pregnancy. The positive oncoFISH cervical test result of 2 nuclei (out of 785 cells examined) with 5 or more copies of 3q strongly suggested the oncogenetic progression of the lesion, thus supporting intervention with a LEEP biopsy. Examination of the biopsy specimen established the presence of a small focus of CIN 3 just
beyond the transition zone with partial involvement of a single endocervical gland. The ability of the oncoFISH cervical test to identify 2 nuclei with 3q gain out of 785 cells examined indicates a high sensitivity for identifying potentially significant lesions and suggests the utility of using this test in helping to assess and manage patients with abnormal cytologic cervical smears, especially when
histologic confirmation is absent or equivocal.