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Poster Session Abstracts

1 Distribution of Arylamine N-Acetyltransferase (NAT2) Genotypes among Omani Arabs, M.O.M. Tanira1, M. Simsek2, K. Al Baloushi3, K. Al Lawatta4, H. Al Barawami5, and K. Bayousmi1, Departments of 1Pharmacology and 2Biochemistry, College of Medicine, Sultan Qaboos University, Al Khod, Oman 123.

Polyomorphic arylamine N-acetyltransferase (NAT2) genotyping was determined among 127 unrelated apparently healthy Omani. The seven known mutations of NAT2 gene namely, G191A, C282T, C341T, C481T, G590A, A803G and G857A were detected using PCR-RFLP analysis. Eleven allele variants (3 alternative) and 30 different genotypes were determined. The commonest alleles were found to be NAT*5B, NAT2*6A and NAT*4 with corresponding alternative) and 30 different genotypes were determined. The commonest genotypes were found to be 5B/5B, 5B/6A, 4/5B, 4/6A with frequencies 0.165, 0.157, 0.118, 0.110 and 0.079 respectively.

2 Multichannel Piezoelectric Gensensor for Detection of Human Papilloma-virus. Wang Jiang-Hua, Fu Wei-Ling, Zuo Qian-yong, Wang Ying-ying, Department of Medical Laboratory, South-Western Hospital, The Third Medical University, ChongQing 400038, P.R. China.

Objective: To establish a methodology for rapid detection and sub-typing of HPV in biomedical samples. Method: We utilized the Piezoelectric Gensisensor(PG) technique, which was a combination of the Piezoelectric Biosensor and Gene Chips to detect bacteria and virus. We used AT-cut, 10 MHz piezoelectric crystals(PEC) for the immobilization of single stranded nucleic acid and the subsequent hybridization reactions. This system is based on detecting small changes in mass on the surface of a PEC which occur upon immobilizing azido containing probe nucleic acid on the surface and hybridizing complementary target nucleic acid to previously immobilized probe. In our work an array of piezorsensors and a computer-controlled system of multichannel measurement and data acquisition construct this instrumentation for multisample clinical testing. The array, a microwell-like plate, is composed of 12 piezorsensor, one of which is used as a reference well, and others are used as sample wells. The frequency shift between each sample and the reference sensor is measured in real-time and saved for later data processing. When reaction between antibody and antigen happen, there will be small changes in mass on the surface of piezoelectric crystal, which will result in frequency decrease of piezoelectric sensor. The frequency decrease depended on the concentration of the IgE . The quantification of total human IgE by means of piezoelectric sensor array technique was compared with conventional radioimmunosorbent test. Result: The piezoelectric sensor array technique can quantitatively detect human serum IgE in the range of 18–310 IU/mL. The correlation between the VDR geno-

3 Application of Piezoelectric Sensor Array Technique for Quantification of Total Human IgE. Fu Wei-ling, Wang Jiang-hua, Department Of Microbiology, University of Mississippi Medical Center, Jackson, MS 39216-4305.

Objective: To establish a methodology for rapid quantification of total human IgE. Method: We utilize the piezoelectric sensor array technique, modifying the piezoelectric crystal with an specific antibody, to detect human IgE. In our work, an array of piezorsensors and a computer-controlled system of multichannel measurement and data acquisition construct this instrumentation for multisample clinical testing. The array, a microwell-like plate, is composed of 12 piezorsensor, one of which is used as a reference well, and others are used as sample wells. The frequency shift between each sample and the reference sensor is measured in real-time and saved for later data processing. When reaction between antibody and antigen happen, there will be small changes in mass on the surface of piezoelectric crystal, which will result in frequency decrease of piezoelectric sensor. The frequency decrease depended on the concentration of the IgE . The quantification of total human IgE by means of piezoelectric sensor array technique was compared with conventional radioimmunosorbent test. Result: The piezoelectric sensor array technique can quantitatively detect human serum IgE in the range of 18–310 IU/mL. The detection limit is 7.1 IU/mL. A total of 28 serum samples are detected by this new method and the results agree well with those given by radioimmunosorbent test, the correlation coefficient is 94%. Conclusion Our results showed that piezoelectric sensor array technique is a accurate rapid and sensitive method for quantification of total human IgE.

4 The Analysis of the Correlation between Vitamin D Receptor Gene Polymorphisms and Bone Mineral Density. Weijing Chu, Xiaoyuan Huang, Yutao Liu, et al., Beijing Ji Shui Tan Hospital, Beijing Institute of Traumatology & Orthopedics, Beijing 100035, P. R. China.

Objective: To investigate the frequency distribution of vitamin D receptor (VDR) genotype in Chinese population and evaluate the correlation between VDR gene polymorphisms and bone mineral density (BMD). Methods: Polymerase chain reaction (PCR) and restriction analysis were used to determine VDR genotypes in 148 Chinese women. Their L2-4 lumbar spine BMD was measured by dual-energy X-ray absorptiometry (DEXA). Results: The frequency distribution of the VDR genotype was: BB, 2.7%; Bb, 8.1%; bb, 89.2%. The BMD in premenopausal women of different genotypes were 1.233±0.105g/cm2 (BB), 1.290±0.141g/cm2 (Bb) and 1.185±0.130g/cm2 (bb) respectively. The BMD was significantly lower in postmenopausal women than premenopausal women (p<0.01). The incidence of osteoporosis in postmenopausal women is 34%. Conclusion: The frequency distribution of the VDR genotype in Chinese peri- and postmenopausal women were consistent with those of a Western women in China. The correlation between the VDR genotype and BMD was not found.

In many instances, multiple analysis of a single sample is desirable. However, when the sample size or content is limited, this becomes difficult or impossible. Multiplex amplification such as PCR, and particularly Isothermal Amplifications, is essential in developing a large number of assays simultaneously. The Xtra Plex approach is a novel method for boosting the amount of input starting material for distribution among large numbers of individual analyses. It involves multiple, sequential rounds of Limited, Multiplex amplification in a closed contained system such as the Taqman or Cobas analyzers. Additionally, the potential for RNA contamination is much easier to control than amplification method, especially useful when sample size is limited. In addition to exponential increase in RNA, the reverse transcriptase activity effects a moderate increase in cDNA copies. Here we describe a combination of Limited, Multiplex Amplification from archived nucleic acid bound irreversibly to a solid-phase binding material (Xtra Amp tubes) as the boosting method. Output from these initial rounds of multiplex amplifications are distributed into the several, single, subsequent analyses. This has been demonstrated with PCR and NASBA. Using 25 distinct PCR primer pairs targeting human genes, five sequential rounds of five primers each have been performed using this technique with no loss of performance relative to simplex reactions. In a separate experiment, 20 primer pairs have simultaneously been used in a single booster multiplex from one extraction in an Xtra Amp tube with identical results. In another set of experiments, twelve sequential rounds of booster/secondary PCR amplifications have been performed from the same blood extraction in the same Xtra Amp tube targeting the same gene and using the same primer set.

Ten simultaneous, NASBA reactions were performed in a single booster multiplex, with the product from this booster reaction supplying ten subsequent simplex NASBA reactions. Eight of these ten reactions worked with no optimization for multiplexing. In two sequential rounds of five primer set multiplex NASBAs from the same extraction in an Xtra Amp tube gave nine of ten successful secondary simplex NASBA reactions. By taking advantage of the irreversible binding capacity of Xtra Amp tubes for Nucleic Acids, and by performing multiple booster amplifications followed by numerous secondary simplifies amplifications, a very large number of assays can be performed on a very limited or precious sample.

To Design a Super-Sensitive Transcription Mediated (TMA) and Polymerase Chain Reaction (PCR) Dual Amplification Assay for the Detection of Hepatitis C Viral RNA Molecules, Venkatakrishna Shyamala, Bruce H. Phelps, and David Y. Chien, Chiron Corporation, Emeryville, CA 94608.

The polymerase chain reaction (PCR) has proven to be a powerful nucleic acid amplification method to generate high yields of DNA. The Transcription Mediated Amplification (TMA) technology has demonstrated its ability to amplify nucleic acids to generate high yields of both RNA. Both techniques are designed to amplify specific nucleotide sequences in an exponential manner, thus providing a basis for sensitive detection of the nucleotide sequence. In the TMA, in addition to exponential increase in RNA, the reverse transcriptase activity effects a moderate increase in cDNA copies. Here we describe a combination of TMA and PCR methods to generate an extremely efficient and sensitive amplification method, especially useful when sample size is limited. In addition, the potential for RNA contamination is much easier to control than for DNA. Detection of the PCR amplification product can be performed in closed contained systems such as the Tagman or Cobas analyzers.

We demonstrate the efficacy and sensitivity of this assay for the detection of HCV RNA molecules in infectious plasma. The TMA reagents were used to capture HCV RNA, to reverse transcribe and generate high yields of specific RNA molecules and concomitant intermediate yields of cDNA. An aliquot of this was used as starting material for PCR amplification. As few as 3 copies of HCV RNA could be detected using as little as 0.3 ml of plasma for the TMA amplification.

This method should be applicable for detection, quantitative estimations and analytical studies with a different fluorescent technology technique require sensitive detection of diagnostic agents; quantitative analysis in clinical trials, in gene expression analyses, and in analytical systems involving DNA typing for forensic analysis, archeological sampling and diagnosis. For analytical studies requiring resolution of amplified DNA by agarose gels for forensic, archeological, and single nucleotide polymorphisms, the usual precautions to prevent contamination should be observed.

Homogeneous PCR Screening Assay for Human Parvovirus B19. Jay Ji1, Xiuli Chen1, Mark Manak1, Sandra Randall1, and Glenn Nardone2, Biotech Research Lab, Gaithersburg, MD 20877; 2Intergen Company, Gaithersburg, MD 20877.

A rapid, reliable and quantitative DNA test for human parvovirus B19 is needed by the blood industry to prevent B19 transmission through blood and plasma derived products. Quantitative determination of parvovirus B19 DNA is also important for monitoring viral load in infected patients. We have developed a PCR-based closed-tube fluorescent assay for the detection of human parvovirus B19 in archived nucleic acid blood and plasma by broad range of pathogenic HPV genotypes to aid in the management of cervical dysplasia.

Detection of High-Risk Human Papillomavirus with Peptide Nucleic Acid Probes. M.A. Cobenfords, J. Williams, C. Chapron, and B.B. Lentrichia, 1Molecular Diagnosis Centre, Department of Laboratory Medicine, National University Hospital; 2Department of Pathology, National University of Singapore.

The human coagulation factor VII (FVII) gene on chromosome 13q34 contains 9 exons and 8 introns spanning 12.8 kb. Elevated FVII activity (FVIIc) in blood has been shown to be associated with increased risk of ischaemic cardiovascular diseases (CVD), thrombosis and myocardial infarction (MI). The R353Q (MafQ) single nucleotide polymorphism in the hypervariable region 4 of intron 7 has been reported to be strongly associated with plasma FVIIc in healthy individuals from the UK (p<0.0001). The single nucleotide polymorphism (G→A) at codon 353 leads to replacement of arginine(R) with glutamine(Q). We had previously reported the allele frequencies of the Q allele to be 0.04, 0.10 and 0.22 for Chinese, Malays and Indians, respectively. The higher allele frequencies of the Q allele, which predisposes to lower levels of FVIIc, in Chinese, Malays and Indians, respectively. Experiments showed the higher incidence of cardiovascular diseases among them. Another polymorphism in the hypervariable region 4 of intron 7 has been reported associated with increased FVIIc. There are 8 of these noncoding regions with tandem repeats of 37 bp monomers. H6 (443 bp with 6 monomers) is the most common variant, H7 (480 bp with 7 monomers) is a less frequent variant, and H5 (405 bp with 5 monomers) is rare. The H1H7 and H6H5 genotypes are associated with the highest risk of MI, followed by the H6H6, H5H7 and H7H7 genotypes (p<0.001). Results: We genotyped 291 healthy Singaporeans (99 Indians, 100 Malays, 92 Chinese). Our results showed that the most frequent genotype was H6H7 (Indians:48.5%, Malays:50%, Chinese:52.3%), followed by H6H6 (Indians:33.3%, Malays:26%, Chinese:26.1%) and H7H7 (Indians:16.2%, Malays:22%, Chinese:18.5%). We also found 2 novel variants which were >480 bp long in 2 Chinese individuals with genotypes H6H9 and H7H10 (heterozygotes with 9 and 10 repeated monomers, respectively, as confirmed by DNA sequencing). The functional significance of these new variants is not known.

Microarray technologies have revolutionized the analysis of gene expression and DNA sequence. In microarray applications, nucleic acids are typically labeled with fluorescent dyes and hybridized to cDNAs or oligonucleotides configured on a solid surface. Fluorescent signals are detected and interpreted using a confocal laser fluorescence scanner and image analysis software. In practice, limited sensitivity, poor signal reproducibility and expensive instrumentation often encumber the performance and accessibility of many microarray systems.

To address these microarray system performance issues, we have applied Resonance Light Scattering (RLS) particle technology, a novel, ultra-sensitive and simple signal generation and detection system (1,2, to microarrays. Submicron RLS gold and silver particles of uniform dimension (20 nm diameter range) scatter incident white light to generate highly intense monochromatic light. The colored light signal generated by an RLS particle is 10^4 to 10^6 times greater than the signal emitted from sensitive fluorophores. RLS signal does not photobleach and different colors and intensities of the generated scattered light can be accurately predicted by application of algorithms derived from electromagnetic radiation theory. On solid surfaces, RLS signal can be detected by the unaided eye at particle densities as low as 0.01 to 0.003 particles/μm². RLS particles can be derivatized with a variety of molecules for analytical bioassays including proteins, antibodies, small molecule ligands and nucleic acid probes. Once derivatized, RLS particles are highly selective for detection of specific biomolecular targets. Simple detection instrumentation consisting of an illumination source, configured optics, CCD-video camera and image analysis system has been developed for microarrays. The optical properties and practical application of RLS particles for microarray systems will be presented.


Identification of Gene Amplifications in Tumor Specimens by Genomic Microarrays. J. Zhao1, J. Rothi1, B. Bode-Leśniewska1, Ph. U. Heitz2, and P. Kompimolin1

Department of Pathology and 2Division of Cell & Molecular Pathology, University of Zurich, Switzerland.

Detection and mapping of genes amplified in tumors is important for the basic understanding of tumorigenesis and the prediction of the tumor biological behavior. Comparative genomic hybridization (CGH) was developed for genome-wide analysis of DNA sequence copy number alterations in a single experiment. CGH using metaphase chromosomes, however, has a limited mapping resolution. Microarray technology enables hybridization to an array of mapped known genes instead of metaphase chromosomes, offering an ideal means of achieving increased mapping resolution and directly associating copy number alterations with the initiation and development of a tumor. In this study, we used a microarray-based CGH technique to search for amplified genes in 15 tumor specimens including 6 pulmonary artery intimal sarcomas (PAIS) and 9 adrenocortical tumors (ACT). Which were selected for based on our CGH studies on a series of 43 tumors. Tumor and normal genomic DNAs, which were labeled with the fluorophore dyes Alexa488 (green) and Alexa594 (red), respectively, were cohybridized to microarrays containing 58 oncogene targets (Vysis, Downer Grove, IL). In CGH analyses, all 6 PAISs consistently showed gains or amplifications of 12q32–q15. Of the 9 ACTs, 2 exhibited amplifications of this region, too. Using microarrays, we demonstrated that, among genes located on 12q32–q15, SAS/CDK4 were amplified in 6 PAISs, MM2D and GLI5 in 4 and 5 PAISs, respectively, and that SAS/CDK4 and MM2D were highly increased in the 2 ACTs. PDGFRα (located on 4q12) amplification was identified in 4 PAISs using microarrays. Amplification at the corresponding chromosomal region of this gene was detected only in 1 PAIS by conventional CGH. Additional gene amplifications that were revealed in 1 or 2 PAISs by CGH, Additional gene amplifications that were revealed in 1 or 2PAISs by CGH, included ESR1, ESR2, E4 and E5. These isoforms depending on their binding affinity to LBD receptor cause accumulation of lipids in the circulation and predispose an individual to CHD. We investigated the role of Lpa (apo E) and Liprotein(a) [Lp(a)] as Risk Factors for CHD: The Indian Study. T. F. Ashavaid, Seema P. Todur, Research Laboratories, P. D. Hinduja Hospital & Medical Research Centre, Mumbai, India.

Apolipoprotein E (apo E) and Lipoprotein(a) [Lp(a)] are considered as risk factors for CHD. Lp(a) is a major risk factor in the development of atherosclerotic lesions. Lp(a) levels are estimated by ELISA which used antibodies against apolipoprotein B component of Lp(a). Genomic DNA was PCR-amplified using primers specific for the polymorphic apo E locus coding amino acids 112 and 158. Restriction enzyme digestion of the amplified DNA with HpaI and size-fractionation on 8% polyacrylamide gel was carried out for identification of the genotypes. When compared against the controls Lp(a) levels were found to be statistically significant in Group A (x² = 5.48, df = 1, P < 0.05). Apo E allele frequencies in the order e2, e3 and e4 were found to be 0.010, 0.880, 0.110 for Group A, 0.080, 0.830, 0.090 for Group B, and 0.040, 0.920, and 0.040 in the control group respectively. (4 allele frequency in Group A against e2 + e3 was found to be statistically significant when compared against controls (x² = 5.4, df = 1, P < 0.05). In Group C, e2 and e4 allele Lp(a) levels were observed in 4 and e4 alleles in 2 subjects. The presence of elevated Lp(a) levels in subjects at high risk allows Lp(a) to be used as a potential risk factor for CHD. The association of Lp(a) and CHD was not statistically significant. However, our study supports the hypothesis of increased prevalence of e4 allele with elevated cholesterol that may be one factor contributing to susceptibility to CHD.

A Novel Enzymatic Approach for Nucleic Acid Mutation Detection: Applications to Congenital Adrenal Hyperplasia. Natalie Belz1, Anthony Killeen2, Daniel Kephart3, and John Shultz1

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A novel enzymatic approach for nucleic acid mutation detection (READIT™—Reverse Enzyme Activity DNA Interrogation Test) has been developed, which is capable of analyzing a wide variety of sequence variations including single nucleotide polymorphisms (SNPs), deletions, insertions and gene fusions. The technology exploits the ability of READase™ polymerase to catalyze pyrophosphorolysis, the reverse of polymerization, in which the addition of inorganic pyrophosphate across the terminal phosphodiester bond liberates deoxynucleotidetriphosphates (dNTPs). The released dNTPs are then converted to ATP in the presence of ADP and READase™ kinase, and the ATP is quantitated using firefly luciferase. The analysis of bi-allelic polymorphism utilizes two interrogation probes. The first probe is perfectly homologous to one form of the template (i.e. wild type) and is positioned such that its 3' end corresponds to the site to be interrogated. A second interrogation probe that hybridizes to the same template position, but whose 3' end corresponds to the alternative (i.e. mutant) form of the template is used. The perfect hybrid formed by the wild type and mutant interrogation probe and their respective templates will then serve as substrates for READase™ polymerase-mediated pyrophosphorolysis. CAH is a relatively common genetically complex autosomal recessive disorder in which mutations in the steroid 21-hydroxylase gene (CYP21) disrupt normal adrenal hormone synthesis. In addition to the expressed form of the CYP21 gene, a 95% homologous pseudogene (CYP21P) resides in tandem to CYP21. A variety of mutations are transferred from the pseudogene to the expressed gene by gene conversion events. These mutations include small deletions, point mutations, and various structural rearrangements. Approximately 20% of CAH is due to large gene deletions, in which an approximately 30 kbp region of the 21-hydroxylase locus is deleted. A PCR and READIT™ strategy has been developed to detect the 9 most common mutations previously published for CAH patients (which result in 95% of all SNP mutated CYP21 alleles). CYP21 is specifically amplified in two overlapping fragments and the presence of any mutation detected in a multiplex interrogation assay. The exact mutation present is then deconvoluted by further interrogation assays for each individual mutation site.


The detection of target nucleic acid hybridized to a complementary capture probe is of central importance in genomic research and pathogen diagnostics. DNA sequencing on microarray chips, gene expression analysis, cancer diagnostics, and molecular diagnosis of infectious agents are just a few examples of methodologies that require such detection. Current methodologies require attachment of enzymatic, fluorescent, chemiluminescent, or radioactive labels to the hybridized target. Furthermore, these methods require amplification of rare targets to detect low copies of a specific nucleic acid or result in protein truncation. Approximately 20% of CAH is due to large gene deletions, in which an approximately 30 kbp region of the 21-hydroxylase locus is deleted. A PCR and READIT™ strategy has been developed to detect the 9 most common mutations published previously for CAH patients (which result in 95% of all SNP mutated CYP21 alleles). CYP21 is specifically amplified in two overlapping fragments and the presence of any mutation detected in a multiplex interrogation assay. The exact mutation present is then deconvoluted by further interrogation assays for each individual mutation site.
The format currently in use is a 200 micron diameter ITO electrode on which an oligonucleotide capture probe is self-assembled. A nucleic acid target is allowed to hybridize to the capture probe and, after washing, Universal FRET Primers. The assay is then amplified and signal acquired from the catalytic oxidation of guanine. Use of hypoxanthine, a 1000 fold less electrochemically active base analogue of guanine, minimizes background current contributions from the capture probe. Using the 200 micron diameter electrode, 40 attomoles of a synthetic 21-mer oligonucleotide containing 5 guanines generated a signal of approximately 100nA over background. The specificity and sensitivity of the system is being optimized using synthetic oligonucleotide capture probes and targets, PCR products and mRNA from cultured cells.

The system is being optimized using synthetic oligonucleotides, PCR products, and mRNA from cultured cells. For example, successful hybridization has been achieved at the electrode using peptide nucleic acid probes, with 30% of the RNA target captured in 2 hours. The detector system can be readily multiplexed, and we are designing a 96 well microtiter plate with seven individually addressable test electrodes in each well. This design will allow the detection of five different sequence specific nucleic acid targets plus two controls per well or up to 672 analyses (480 samples and 192 controls) per plate.

**Amplifluor™ Detection System for Viral Target Screening and Quantitation.** Sandra K. Randall, Glenn A. Nardone, Jay Ji, Nate Lawrence, and Mark Mancini 1

Homogeneous and sensitive PCR based fluorescent detection assays suitable for high throughput viral screening are needed for screening blood and blood products. The desired closed-tube PCR assay for most viral load testing requires linearity in the range of 100 copies/ml to 10^10 copies/ml, while assays for high throughput screening must be cost effective with sensitivity below 100 copies/ml of blood. Using the Amplifluor™ technology, we developed a competitive assay for the simultaneous detection of HBV DNA with an Internal Control (IC) that is suitable for quantitative real-time analysis with the capability for high throughput viral screening by endpoint analysis.

The Amplifluor™ system is based on the incorporation of energy transfer hairpin primers into the amplification products. During PCR, there is a common primer and two different gene specific Amplifluor™ primers. The excitation/emission wavelengths of the two Amplifluor™ primers allow for the simultaneous amplification and detection of HBV (FAM) and internal control markers, and individual genotyping panels can be constructed by selection from a library of characterized eTags. The modularity of this strategy enables typing multiple samples with respect to a single genetic target ApoE and CYP2D6.

**Development of a New Assay Format for High-Throughput, Multiplexed SNP Genotyping.** Alexander P. Sass1, Elaine S. Mansfield2, Carmen Virgos3, Wendy Levine1, Milton F. Chaves1, Stephen J. Williams1, Sheela Muley1, Shared Ferguson1, Jing Wei1, Suzan Leon1, Tracy Matray1, Vincent Hernandez1, Ken J. Livak2, Penny Dong2, Sharat Singh1, and Maureen Cronin 1

DNA extraction using the Qiagen BioRobot followed by PCR and detection enables rapid screening and quantitation of samples within a 4-hour period. HBV strains from human plasma samples were extracted and analyzed by both the `read-time' and `endpoint-time' analysis. The former showed a low background signal and a viral titrer consistent with 100 copies/ml of blood. The Amplifluor™ assay serves as a model development of sensitive viral assays compatible with screening of blood or blood products.

**A New Method for SNP Genotyping Based On Allele-specific PCR With Universal FRET Primers.** Y. Khripin1, M. V. Knezevic2, S. H. Hu2, K. Lueders2, D. Hamer3, Intergen, Gaithersburg, MD; 2NIH, NCI, Bethesda, MD.

We have developed a new method for high throughput genotyping of single nucleotide polymorphisms (SNPs). The technique involves PCR amplification of genomic DNA with a single SNP containing FRET primers that introduce priming sites for universal fluorescence resonance energy transfer (FRET) primers. The output of red and green light is conveniently scored using a fluorescence plate reader. The assay was validated on nine model SNPs representing all four possible types of single nucleotide substitutions. The new method is well suited for high throughput, automated genotyping because it requires only one reaction per SNP, it is performed in a single tube with no post-PCR handling, the same FRET primers are used for all analyses, and can be used to design a large number of independent SNP assays. It shows the utility of candidate gene analysis, genome-wide scans, and medical diagnostics. The method has been used to genotype 6 candidate genes for nicotine dependence and personality traits (DRD1, HTR2C, MAOA, HTR2A, HTR1B, DRD2, CHRNA2, CCK) on 2000 individual DNA samples.

**The Importance of Considering MTHFR Genotype and Serum Folate Together with Homocysteine in Assessing the Cardiovascular Risk Attributable to Hyperhomocysteinemia: A Meta-analysis.** P. Mastandrea, Laboratory Medicine, Ospedale Rizzoli, Ascoli,Italy.

Hyperhomocysteinemia (Hyp) is an independent factor of cardiovascular risk (CVR). A thermostable variant (C677T) of methylenetetrahydrofolate reductase (MTHFR) shows a decreased activity. Hyp increases because of decreased methionine synthesis. As Brattstrom indicated in his meta-analysis, reductase (MTHFR) shows a decreased activity. Hyp increases because of decreased methionine synthesis. As Brattstrom indicated in his meta-analysis, reductase (MTHFR) shows a decreased activity. Hyp increases because of decreased methionine synthesis. As Brattstrom indicated in his meta-analysis, reductase (MTHFR) shows a decreased activity. Hyp increases because of decreased methionine synthesis. As Brattstrom indicated in his meta-analysis, reductase (MTHFR) shows a decreased activity. Hyp increases because of decreased methionine synthesis. As Brattstrom indicated in his meta-analysis, reductase (MTHFR) shows a decreased activity.
heterogeneity tests for a) and b) and the Student t-test for [3] [4] were positive. The heterogeneity test for c) and the Student t-test for [5] were negative. In conclusion, fHcy heterogeneity can be avoided by adjusting data to fHcy determinants. Perhaps it is necessary to consider the need for fMTHFR and MTHFR together in assessing the fHcy-related CVR. Nevertheless, the difference between cases and controls can not be explained by folate and MTHFR levels only, then we should research other determinants.

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A new membrane filter-based method for the rapid and simultaneous detection, identification, and enumeration of Escherichia coli using peptide nucleic acid (PNA) probes and a laser scanner within one working day is presented. PNA probes are DNA mimics with a polyamide backbone to which the individual nucleobases are attached. Their ability to hybridize to complementary nucleic acid targets with high specificity and rapid binding kinetics have opened new possibilities for molecular diagnostic assays. The method is based on traditional membrane filtration technique for capturing microorganisms from large sample volumes. Following sample filtration, the membrane filter was incubated for 5 hours on growth medium to allow formation of micro-colonies. The micro-colonies were then analyzed directly in situ hybridization using fluorescencely-labeled PNA probes targeting a species-specific 16S rRNA sequence. Using an array scanner with 5 mm resolution, the entire surface of a 25 mm membrane filter was analyzed for the presence of fluorescent micro-colonies within 8 minutes, thereby providing rapid and simultaneous detection, identification, and enumeration of E. coli. A rapid phenotypic susceptibility assessment of E. coli has also been performed by the use of antibiotics in the growth medium. This new method is promising for a variety of different applications within clinical and industrial microbiology.

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Protein Expression Profiling on Microarrays by Rolling Circle Amplification. B. Schweitzer, S. Willshire, J. Lambert, M. Mullenix, S. Kingsmore, Molecular Staging Inc.

Microarray-based mRNA expression profiling is a powerful functional genomics tool, but suffers from a major limitation of discordance with protein expression levels. Conventional approaches to protein measurements are either too expensive or lack throughput for simultaneous monitoring of expression levels of many proteins in parallel. Rolling Circle Amplification (RCA) is emerging as the signal amplification method of choice for DNA and RNA microarrays, since RCA uniquely allows target recognition, amplification, and detection in situ on the microarray. We have recently developed an adaptation of rolling circle amplification (RCA) for the detection of antibody bound to antigen, termed "immunoRCA". In immunoRCA, the 5' end of an oligonucleotide primer is attached to an antibody; thus, in the presence of circular DNA, DNA polymerase, and nucleotides, the rolling circle reaction results in a concatamer of DNA that can be detected by hybridization to the antibody. The amplified DNA can be detected by hybridization of complementary oligonucleotide probes. ImmunoRCA, therefore, represents a novel approach for signal amplification of antibody-antigen recognition events on microarrays. We have previously shown that ImmunoRCA enables analyte detection on immunomicroarrays with a sensitivity limit of 100 zeptomoles and a dynamic range of greater than 4 logs using a conventional microarray scanner (Schweitzer et al., Proc. Natl. Acad. Sci. (USA), in press.). Results will be presented from a retrospective study involving 80 allergic patients that demonstrate that the clinical accuracy of ImmunoRCA detection of allergen specific IgE on microarrays of allergen extracts is comparable to conventional immunosay when compared to the skin prick test. We have also constructed a microarray consisting of approximately 50 different antibody-to-cytokines, chemokines, and growth factors. Using ImmunoRCA for signal amplification, pg/ml sensitivity has been demonstrated for simultaneous detection of multiple proteins on this array. A two-color adaptation of ImmunoRCA has also been developed that permits simultaneous measurement on a microarray of protein levels from two samples in a manner analogous to RNA expression profiling.

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Post-PCR Detection and Discrimination of the CYP2C9*1, CYP2C9*2, and CYP2C9*3 SNPs Using Gene Probes. Marc Goldford, John Backus, and Mark Triscott, Sigma Diagnostics, St. Louis MO.

The cytochrome P450 enzyme C29 segregates into several alleles due to single nucleotide polymorphisms (SNPs). This work undertook the development of a simple, inexpensive, and timely diagnostic technique for the CYP2C9*1 wild-type and the CYP2C9*2 (nt416) and CYP2C9*3 (nt1061) mutant SNPs using microtiter plate capture of biotinylated amplicons with subsequent identification by fluoroeinated DNA probes.

Locus Probes
CYP2C9*1 Wang
(fwd)GGTATAGAAGCGAACAAGAA
(rev)GGCTTGGTTTCTTCACATC
CYP2C9*2 Wang
(fwd)GGTATAGAAGCGAACAAGAA
(rev)GGCTTGGTTTCTTCACATC
CYP2C9*3 Wang
(fwd)GGTATAGAAGCGAACAAGAA
(rev)GGCTTGGTTTCTTCACATC

The genotypes of 25 random donors were first determined by restriction endonuclease/ gel electrophoresis using the method and primer sets of Wang (Pharmacogenetics 5: 37, 1995). One homozygous mutant, seven heterozygotes, and seventeen homozygous wild-types were found for nt416, and two heterozygotes and twenty-three homozygous wild-types for nt1061. These DNA samples were then amplified with primers after Nasu (Pharmacogenetics 7: 405, 1997) for nt416 and after Steward (Pharmacogenetics 7: 361, 1997) for nt1061 detection. Both reverse primers were 5' biotinylated in this study. Amplicons were bound in duplicate to streptavidin coated plates, denatured, and washed to remove the unmodified complementary DNA strand from each well. One well of each pair was then hybridized with a 15 bp fluoroeinated wild-type probe solution containing unlabelled competitor mutant probe, and the other well with a 15 bp fluoroeinated mutant probe solution containing unlabelled competitor wild-type probe. After hybridization OD 450 was developed with anti-fluorescin antibody HRP conjugate and TMB substrate. The ratios of WT/Mutant OD 450 were calculated, and clearly segregated to >10 (homozygous wild-type, 0.7 to 2.0 (heterozygote), or <0.2 (homozygous mutant). The genotypes determined by probe assay replicated the results determined by RE/GE for both loci, demonstrating 100% sensitivity and 100% specificity. This plate capture/probe detection method requires minimal expensive reagents and can be performed in two to three hours, can be automated with readily available microtiter plate instruments, and can simultaneously discriminate multiple loci in a "macroarray" format.

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Peptide Nucleic Acids (PNAs) are a unique class of molecules that are DNA mimics and demonstrate improved hybridization kinetics and specificity. The non-charged backbone of PNA allows hybridization to be performed under low salt conditions that are denaturing for RNA secondary structure but favorable for PNA-RNA hybridization. rDNA is a well established target for molecular diagnostic assays due to its high cellular abundance, universal distribution, and use as a phylogenetic marker. Using the publicly available sequence databases, species-specific RNA sequences have been identified and complementary PNA probes synthesized. The PNA probes were labeled with fluorescent dyes and used to develop a fluorescent in situ hybridization method (PNA FISH) for identification of microorganisms. The PNA FISH method uses universal fixation and hybridization conditions for gram-negative and gram-positive bacteria, as well as yeasts. PNA probes labeled with different fluorescent dyes were applied individually, or in a multiplex assay in which up to four different labeled PNA probes were used simultaneously with a mixed population of cells. The fluorescent dyes which have been used include fluorescein, cy 3, cy 5, coumarin, rhodamine, and alexa dyes. Examples using species-specific probes for Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa, and Staphylococcus aureus, will be shown in both single and multiplex assays.

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Transcriptome Analysis Based on Isothermal Sequence Amplification. Kiyoshi Yashawaka and Takahiko Ishiguro, Tokyo Research Laboratories, Tosoh Corporation, Kanagawa 252-1123, Japan.

As the full-length sequences of genomes of several species including human genome of 3 billion bp come into view, the post-genomic analysis has already started. Despite the advances in computational prediction of unidentified genes, it is still difficult to accurately identify genes from genomic sequences themselves, and transcriptome analysis should be based on the experimental data.
In this study, we have devised a novel method to identify genes throughout genomes based on isothermal sequence amplification. The complete genome or its part is divided into fragments of approximately 100 to 200 bp. Based on the nucleotide sequences of each fragment, sense and anti-sense DNA primers are synthesized. If the amplification occurs using the primers, the fragment is identified to be the entire transcriptional region. In the model experiments, the 900 bp genomic sequences of the yeast *pichia pastoris* was divided into 5 fragments of 180 bp. The transcriptional region of the sequences had been already known. By the isothermal RNA amplification method, TRC (transcription-reverse transcription amplified conversion), we found that the amplification was specific to the fragments that were entire transcriptional regions. We also demonstrated that the amplification could be improved by the INAF (intercalation activating fluorescence) probe in the course of the TRC reaction. It was found that the sensitivity in TRC was 10 times higher than that in RT-PCR. Genomic DNA contamination led to RT-PCR amplification even in the absence of the targeted mRNA, while such undesired amplification was not observed in TRC. It was also found that 30-minute incubation was enough for the detection of the targeted mRNA in the TRC reaction whereas 2 to 3 hours were required in RT-PCR.

The present results suggest that the fluorescence real-time monitoring of isothermal sequence amplification with INAF probes provides a widely applicable method for the upcoming transcriptome analysis and its automation.

**Evaluation of Semi-Automated Hepatitis C RNA Quantitative Assays Using QIAGEN BIOROBOT RNA Isolation and COBAS AMPLICOR HCV MONITOR Detection**

Russell E. Baumann and Michael A. Lewinski, Quest Diagnostics' Nichols Institute.

An automated viral RNA isolation platform (QIAGEN BIOROBOT 9604) has been optimized, combined with the COBAS AMPLICOR HCV MONITOR v2.0 test, and evaluated performing semi-automated hepatitis C (HCV) viral load determinations with clinical specimens. The QUEST HCV RNA v1.0 protocol, a modified version of QIAGEN's QIAamp 96 Viral RNA protocol, automates the preparation of RNA in a manner equivalent to the COBAS AMPLICOR HCV MONITOR v2.0 manual procedure. A recently validated second generation protocol (QUEST HCV RNA v2.0) additionally automates the transfer of RNA to the COBAS' circular A-rings. The first generation assay (Quest v1.0) showed a high correlation (900 to 1,000,000 HCV copies/ml). Patient viral loads determined using the first generation assay correlated well with the manual HCV MONITOR v1.0 test (r² = 0.98, n = 109). The second generation assay (Quest v2.0) showed a high correlation (500 to 1,000,000 HCV copies/ml). Patient viral loads determined using the second generation assay correlated well with the first generation test (r² = 0.99, n = 135). Mean inter-assay precision, calculated from results for the COBAS Low and High Positive and BHI HCV Accurun Series 300 controls, was 33% (n = 179) for the first generation assay and estimated at 26% (n = 12) for the second generation assay. In comparison, COBAS v2.0 runs performed using manually prepared RNA had mean precision estimates of 32% (n = 5)


Pyrosequencing™ is a real-time sequencing method based on synthesis detection. Pyrophosphate (PPi), released during nucleotide incorporation by DNA polymerase, is detected as light produced by an enzyme cascade system. Pyrosequencing AB (Uppsala, Sweden; Department of Genetics and Pathology, University of Uppsala, Uppsala, Sweden.

Pyrosequencing™ is a real-time sequencing method based on synthesis detection. Pyrophosphate (PPi), released during nucleotide incorporation by DNA polymerase, is detected as light produced by an enzyme cascade system. Pyrosequencing AB (Uppsala, Sweden) recently introduced the PSQ 96™ System, in which 96 different Single Nucleotide Polymorphism (SNP) samples are analyzed in parallel in less than 15 min. All reagents needed for performing a Pyrosequencing Analysis in the System are provided in an SNP Reagent Kit (enzymes, substrates and nucleotides), and dedicated SNP software delivers genotype and a quality assessment for each sample.

**Melting Curve Analysis of SNPs (McSNP): A Simple Gel-free Low-Cost Approach to SNP Genotyping and DNA Fragment Analysis.**

Joshua M. Abbot (University of Texas-Houston) and Mark. D. Shriver (Penn State University).

In order to realize the full potential of SNPs it is essential to develop practical, accurate, and high-throughput methods to genotype them. Although a number of high-throughput SNP genotyping assays have been developed, including the oligonucleotide ligation assay (OLA), minisequencing, TaqMan, and dynamic allele specific hybridization (DASH), the requirements of simplicity and low-cost have not been adequately met. To address these issues, we have developed a method that we refer to as Melting curve SNP (McSNP) genotyping, which is less expensive ($0.12/genotype reagent cost) and simpler to use than existing high-throughput genotyping assays. McSNP combines a classic approach for discriminating alleles, restriction enzyme digestion, with a newer method for detecting DNA fragments, melting curve analysis (MCA). MCA is performed by slowly heating DNA fragments in the presence of the double stranded DNA (dsDNA) specific fluorescent dye SYBR green I. As the particular fragment is heated through its melting temperature (Tm), fluorescence rapidly decreases due to denaturation and detection.

**Improved Method for Factor V Leiden Typing by Polymerase Chain Reaction and DNA Sequencing.**

C.A. Almeida, M.O. Ribeiro, C. Carvalheira, E.F. Andrade, E.M. Correia, M.H. Teixeira, I.B. Fellowes, F.M.S. Leite, Laboratorios Medicos Dr. Sergio Franco, Setor de Biologia Molecular, Río de Janeiro, Brazil.

Factor V Leiden is a single-point mutation in the factor V gene (G1691A) that incorporates arginine by glutamic acid at amino acid residue 506 (R506Q). This substitution prevents a peptide bond in the coagulation molecule from being cleaved by activated protein C (APC). During normal
and thus suggest higher fidelity. Performing Mg2+-activated RT-PCR with the wild-type enzyme. These results suggest that the mis-incorporation rate is lower for the Mg2+ complex.

The Factor V Leiden mutation has been shown to be the most common genetic risk factor for venous thrombosis. This mutation is a single nucleotide polymorphism (SNP) at position 1691 of exon 10 of the gene. This SNP is a G/A transition at codon 506, which substitutes Arg/Gln at the protein. The HCV 5' NCR of 244pb. Amplification products were denatured by adding 50 m of amplification mix. 140 l of 1M Tris-HCl (pH 7.5) and 5 l of the DNA polymerase into one tube of the amplification reaction mix. The mean weekly warfarin dose (±SEM) for all patients of 36.9 (±1.5) mg was not influenced by either gender or ethnicity (p=m for both), but was significantly affected by age (p=0.0073). The frequencies of CYP polymorphisms 2C9*2 (25/153) 16%, 2C9*3 (22/153) 14%, and 2A6*2 (6/153) 3.9%. There were no gender differences in overall warfarin polymorphism frequency (males 38%, females 29%, p=0.31). However, CYP polymorphisms were less common in African Americans than Caucasians (13.6% vs. 38.9%, p=0.03). Patients with CYP polymorphisms (2C9*2, 2C9*3, or 2A6*2) had significantly lower warfarin doses compared to patients with wild-type genotypes (30.6 (±2.5) mg vs. 40.1 (±1.7) mg, p=0.002). Regression analysis suggested a more linear dose-response relationship with genotype (r2=0.265).

Conclusion: Polymorphisms of CYP2C9 and CYP2A6 subfamilies are common, associated with significant reductions in warfarin dose, and likely account for an appreciable component of the variability in dose-response associated with standard dosing protocols. Ultimately, as the interactions between genetic factors and other variables which influence warfarin sensitivity are more completely understood, the use of rapid genetic testing of CYP to guide warfarin therapy may become a useful adjunct for increasing the safety and efficacy of this drug.

Automated Detection of the Factor V Leiden Mutation Using MALDI-TOF Mass Spectrometry on the MassARRAY System. E. Lau1, J. Leushner2, and M. Pataki3, 1Imaging Sciences and Informatics, 2Institute for Radiology, and 3Dermatology, Vanderbilt University School of Medicine, Nashville, TN. The Factor V Leiden mutation is known to modify the balance of thrombotic and antithrombotic activity in patients. We have performed this analysis using two different methods: MALDI-TOF MassARRAY and direct sequencing.

Method: HCV 5' NCR of 244pb. Amplification products were denatured by adding 50 m of amplification mix. 140 l of 1M Tris-HCl (pH 7.5) and 5 l of the DNA polymerase into one tube of the amplification reaction mix. The mean weekly warfarin dose (±SEM) for all patients of 36.9 (±1.5) mg was not influenced by either gender or ethnicity (p=m for both), but was significantly affected by age (p=0.0073). The frequencies of CYP polymorphisms 2C9*2 (25/153) 16%, 2C9*3 (22/153) 14%, and 2A6*2 (6/153) 3.9%. There were no gender differences in overall warfarin polymorphism frequency (males 38%, females 29%, p=0.31). However, CYP polymorphisms were less common in African Americans than Caucasians (13.6% vs. 38.9%, p=0.03). Patients with CYP polymorphisms (2C9*2, 2C9*3, or 2A6*2) had significantly lower warfarin doses compared to patients with wild-type genotypes (30.6 (±2.5) mg vs. 40.1 (±1.7) mg, p=0.002). Regression analysis suggested a more linear dose-response relationship with genotype (r2=0.265).

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High Temperature Magnesium (Mg2+)-activated Reverse Transcription by Thermotolerable DNA Polymerases. Edward Smith, Alice Wang, Christopher L. Siguer, Nanette Schuman, and Thomas W. Smith, Washington University School of Medicine, St. Louis, MO.

Thermotolerable DNA polymerases have been shown to exhibit reverse transcription (RT) activity, i.e. DNA synthesis from an RNA template. However, with thermotolerable DNA polymerases, efficient RT activity has only been achieved with the DNA template that is used in the reverse transcription associated divalent metal ion activator. The purpose of this study was to determine the difference in DNA polymerase activity between clinical laboratories and with high throughput needs and demands for improved accuracy.

We have performed point mutations in the polymerase domain of rTth DNA polymerase that alter the reverse transcription associated divalent metal ion activator and loaded on a "Microspin S-400 HR" column (Pharmacia Biotech). This step resulted in ambiguities (Ns) in the basecalling. Careful examination of the electropherograms showed that the two sequences were identical, except at the base 1691 of the gene where both peaks, G and A, could be seen at the same level. To the heterozygotes carriers, more than one sequence was present in an electropherogram. This resulted in ambiguities (Ns) in the basecalling. Careful examination of the data showed that the two sequences were identical, except at the base 1691 of the gene where both peaks, G and A, could be seen at the same level.

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position 188, which results in a Pro to Ser amino acid change at position 34 with decreased enzymatic activity. During sequence analysis of Asian samples which possessed the CYP2D6*10 allele, we observed that the C and T peaks at position 188 among many heterozygotes were not uniformly equal in height. We investigated this anomaly using pulse field gel analysis, quantitative cloning, and a quantitative duplication assay. In our analysis of 77 Asian samples, the *10 allele occurred with a frequency of 47% and two thirds of the samples with the *10 allele contained multiple copies of the CYP2D6*10 locus. We demonstrated using pulse field gel electrophoresis that the amplified CYP2D6*10 allele is large enough to contain the entire gene. Therefore persons with the CYP2D6*10 allele may have multiple copies of that allele which may compensate for the decreased enzymatic activity of the *10 allele. This study highlights a situation where genotyping a simple SNP may be complicated by alterations in the genomic organization of the locus.

**Suppression of CYP2E1 Promoter Activity by γ-Interferon and Loss of Response Due To a Naturally Occurring G-35/T Nucleotide Sequence Polymorphism.** Mark W. Linder, Ling Q. Liu, Deborah M. Antonio-Green, and Roland Valdes Jr., Department of Pathology and Laboratory Medicine, University of Louisville School of Medicine.

Cytochrome P450 2E1 (CYP2E1) is a major catalyst responsible for the metabolism of many carcinogens and toxic chemicals including vinyl chloride (VC). We have previously reported that the CYP2E1*7B allele which has a G-35/T polymorphism increases susceptibility to VC-induced angiosarcoma of the liver. Evaluation of the CYP2E1 promoter structure flanking the polymorphic G-35 nucleotide revealed a nucleotide sequence that matches 8 of 9 nucleotides of known γ-IFN-responsive ICAM-GAS element. The objectives of this study are to determine if the CYP2E1 promoter activity is regulated by a γ-IFN-dependent mechanism and to determine the influence of the G-35/T polymorphism on this mechanism. Specific binding of γ-IFN activated nuclear protein to the wild-type CYP2E1-GAS element was demonstrated through the ability of the CYP2E1 sequence to compete with the GAS element of the γ-IFN-responsive ICAM gene for γ-IFN-activated nuclear protein binding. In contrast, the CYP2E1-GAS including the variant T-35 was a poor competitor requiring 2-fold higher concentrations to displace 50% of the γ-IFN-activated nuclear protein from the ICAM-GAS. Through transient transfections of HepG2 cells with chimeric luciferase (luc) reporter gene constructs bearing the native and polymorphic CYP2E1 promoter sequences and subsequent treatment with γ-IFN, we demonstrate a 30% reduction of luciferase activity in cells transfected with wildtype (G-35) CYP2E1-luc reporter gene. However, the suppression response to γ-IFN is abolished in cells transfected with CYP2E1-luc reporter containing the T-35 polymorphism. These data support the authenticity of the putative CYP2E1-GAS element and demonstrate decreased nuclear protein binding with loss of response to γ-IFN as the result of genetic polymorphism of this regulatory domain. (Supported by NIH R01-ES-08953, RV)

**Rapid, Sensitive, Sequence-Specific Detection of Cystic Fibrosis Mutations Using a Thin Film Biosensor, R. Jenison, A. Haeberli, and B. Polisky, BioStar, Inc., 6655 Lookout Road, Boulder, CO 80301.**

We have developed a highly sensitive, inexpensive, and rapid nucleic acid hybridization assay utilizing thin film biosensor technology. The technology allows direct visual detection of molecular thin film formation resulting from the interaction of target DNA sequences with oligonucleotide probes immobilized to an optically coated silicon chip. Signal is generated by hybridization of a labeled detector oligonucleotide to the surface-bound target, which triggers reactions that enzymatically transduce the formation of hybrids on the surface into a molecular thin film. The thin film alters the interference pattern of light on the surface, producing a perceived color change. The composition of the optical layers was designed such that small increases in thickness (5–10 Å) occur in a color range where the human eye is most sensitive. In a model system, using sequences derived from the Hepatitis C viral genome we detected 10 attomol/ml or 6 x 10^10 copies/sample in the presence of human serum in one hour. Further, we were able to specifically detect methicillin-resistant *S. aureus* from a single bacterial colony in a 45 minute assay and we have established an assay for the specific detection of viruses responsible for respiratory tract infections that requires only 10 minutes.

We have applied thin film technology to the development of a panel for mutation detection in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Detection of PCR amplified genomic DNA is performed in a multiplex format with detection in a single microwell. Five primer pairs were mixed for the simultaneous detection of eight mutations. One member of each primer pair was labeled with biotin. Specific capture probes for wild type and mutant sequence were immobilized on a single chip, and hybridization signals were discriminated spatially. PCR-amplified DNA was heat denatured, applied to the chip, and detected with an anti-biotin-HRP conjugate and a precipitating enzyme substrate, which produced a visually detected molecular thin film. The assay was completed in 15 minutes. Genotypes were verified for ten human DNA samples with characterized CFTR mutations.