G40. Development of a Rapid Multiplex PCR Assay for Identification of the Three Common Hemoglobin-Lepore Variants (Boston-Washington, Baltimore, and Hollandia) and Identification of a New Lepore Variant

D.L. Vanhille[1], R.H. Nussenzveig[1], A.M. Agarwal[2], J.T. Prchal[2]
[1]ARUP Laboratories, Salt Lake City, UT; [2]University of Utah/ARUP Laboratories, Salt Lake City, UT.

Introduction: Hemoglobin Lepore (Hb-Lepore) is an abnormal hemoglobin composed of two α-globin chains and two hybrid δβ-globin chains. Unequal crossover of a region spanning the δ- and β-globin genes leads to deletion of approximately 7.4 kb and formation of a fused δβ-globin hybrid. Due to reduced synthesis of the hybrid chain, a β-thalassemia minor phenotype is noted. Homozygosity, or compound heterozygosity, of Hb-Lepore and β-thalassemia can result in thalassemia major or intermedia phenotypes. Three common Hb-Lepore variants have been identified based on their deletion breakpoints: Hb-Lepore Hollandia, Hb Lepore–Baltimore, and Hb-Lepore Boston-Washington. We report here the development of a rapid multiplex PCR assay for identification of the three common Hb-Lepore variants in a single reaction tube format. **Methods:** A total of 30 samples were tested using our novel multiplex PCR assay. Nineteen patient samples with suspected Hb-Lepore, 1 Hb-Lepore Baltimore (Coriell, Camden, NJ), and 10 wild-type samples were analyzed. PCR controls include primers within the common Hb-Lepore deleted region (wild-type allele) and primers specific to the 3'UTR of the LIS1 gene (amplification control). All positive Hb-Lepore samples were bi-directionally sequenced to confirm the breakpoints. **Results:** Testing of the 20 Hb-Lepore samples using our novel multiplex PCR assay, indicated the presence of 14 Boston-Washington, 4 Baltimore, and 2 Hollandia samples. Breakpoints for 19 samples were confirmed by sequencing. The breakpoint for one sample initially identified as Hb-Lepore Baltimore by our PCR assay was not confirmed by sequencing. Instead a novel Hb-Lepore breakpoint between δ31Leu and β50Thr was discovered, which we have named Hb-Lepore ARUP. Of the 20 Hb-Lepore samples, 19 were heterozygous harboring one wild-type and one Hb-Lepore allele, and 1 was hemizygous with deleted δ and β globins on one allele and Hb-Lepore on the other. No Hb-Lepore amplicon was detected in the wild-type control samples. **Conclusions:** While standard diagnostic methods may suffice for identification of many hemoglobinopathies, they cannot be used for routine detection and confirmation of Hb-Lepore. Our novel multiplex PCR assay can confirm a Hb-Lepore, rapidly identify the three common variants, and be used for genotyping. The single reaction tube format, ease of setup, high throughput, and rapid turnaround time make our assay highly useful in the clinical laboratory.

H10. A Pyrosequencing-Based Test for Quantitative Monitoring of the BCR-ABL T315I Mutation

J.A. Schumacher[1], P. Szankasi[1], A.K. Ho[1], T.W. Kelley[2]
[1]ARUP Laboratories, Salt Lake CIty, UT; [2]University of Utah, Salt Lake CIty, UT.

Introduction: CML patients and those with Ph+ ALL who are treated with tyrosine kinase inhibitors (TKIs) may develop therapeutic resistance from mutations at important drug-binding sites in the kinase domain of BCR-ABL fusions. The T315I mutation, known as the "gate keeper", imparts resistance to all currently approved TKIs (imatinib, dasatinib and nilotinib). Patients who develop a T315I mutation often experience disease progression. Currently, there are drugs in development that, in early clinical studies, demonstrate activity in T315I positive patients. Thus, a rapid, sensitive and specific test for identifying and quantifying the T315I mutation would be helpful for on-going patient monitoring and for choosing the appropriate therapeutic option. Pyrosequencing has emerged as a useful technique to detect and quantitate point mutations in many clinically important genes (ie. KRAS, BRAF, and Jak2) and provides the advantages of being sensitive and inexpensive with high throughput. Here, we describe the development and validation of a pyrosequencing-based assay designed to detect and quantitate the BCR-ABL T315I point mutation in CML and Ph+ ALL. **Methods:** 32 samples (10 T315I+, 1 T315F+, 1 F311L+, 20 WT) were subjected to one round of PCR amplification across the BCR-ABL breakpoint followed by a second round of PCR using primers, including one with a biotin label, flanking codon 315. Amplicons were subjected to pyrosequencing of codons 315-316. Quantification was expressed as % mutant. Results: The T315I mutation was detected in 10/10 known T315I+ samples and quantified. The assay also detected a T315F mutation and yielded evaluable codon 315 sequence in a case with a F311L mutation which overlapped with the forward primer binding site. RNA from one T315I-positive case with 100% mutant allele was serially diluted into RNA from a BCR-ABL positive donor without the T315I mutation and reverse transcribed. Analysis indicated a sensitivity of approximately 5%.
Conclusions: We describe a quantitative, sensitive, and inexpensive assay for monitoring of the pan-resistant BCR-ABL T315I mutation.

ID10. A Multi-Tiered RT-PCR Approach to Detection, Subtyping, and Oseltamivir Resistance Testing of Influenza A Virus

R. Rattray, F. Moore, R.D. Press
Oregon Health & Science University, Portland, OR.

Introduction: Influenza virus infections hospitalize approximately 200,000 people in the United States resulting in over 35,000 annual deaths. Rapid detection of these infections is important for timely administration of appropriate anti-viral therapies. Traditional methods of virus detection either have long turn-around-times (culture) or lack detection sensitivity (antibody based methods). Thus, molecular diagnostic techniques that are both rapid and sensitive have become the gold standard in influenza virus diagnostics. Our lab has recently developed a multi-tiered RT-PCR-based testing algorithm to: a) detect influenza virus A & B RNA in respiratory samples (including the pandemic 2009 H1N1 strain), b) subtype positive flu A cases, and c) directly assess the flu A H275Y neuramidase gene mutation conferring oseltamivir resistance. **Methods:** RNA is prepared from respiratory specimens (MagNApure) and subjected to an initial multiplex RT-PCR with primers and FAM-labeled MGB Pleiades probes (Epoch) targeting the matrix gene of flu A & B and the polymerase L gene of RSV. The 3 targets are unambiguously identified by their distinct melting curve profiles on the Lightcycler 480 platform. Flu A positive cases are next subtyped by a second matrix gene RT-PCR (based on Stone et al, 2004) that uses post-PCR melting temperatures to distinguish seasonal H1N1, 2009 pandemic H1N1, and H3N2. Oseltamivir sensitivity is assayed in specimens subtyped as Influenza A H1N1 or 2009 pandemic H1N1 by performing RT-PCR pyrosequencing (based on the protocol published by the CDC) to directly detect the conserved H275Y neuramidase gene mutation. **Results:** We applied this detection/subtyping algorithm to 111 respiratory specimens that were each also tested by another diagnostic method and, for influenza A detection, achieved 99% sensitivity (one potential false negative) and 100% specificity. Oseltamivir-resistant strains of 2009 pandemic H1N1 have recently been described, all with a conserved H275Y neuramidase gene mutation. Each of 8 tested seasonal H1N1 isolates from our freezer archive (from early 2009) had the mutation; however, to date, none of 46 tested 2009 pandemic H1N1 isolates had this mutation. **Conclusions:** With this multi-step detection, subtyping, and resistance testing algorithm, accurate diagnosis and treatment can be quickly given to patients presenting with non-specific respiratory virus symptoms.