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Screening for Philadelphia Chromosome (PhC) by an in-house Reverse Transcription-Polymerase Chain Reaction (RT-PCR)- based assay

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The Philadelphia Chromosome (PhC) is observed in more than 90% of Chronic Myeloid Leukaemia (CML) and in a small percentage of Acute Lymphoblastic Leukaemia (ALL) patients. PhC results from a translocation between the long arms of chromosomes 22 (Bcr locus) and 9 (Abl locus) generating a fusion gene, Bcr-Abl, expressing a protein with elevated tyrosine kinase activity, which in turn leads to the development of leukaemia. Chronic Granulocytic Leukaemia (CGL; i.e PhC diagnosed CML) has a good prognosis and therapy is available, if detected early. In contrast, PhC detected ALL has a bad prognosis indicating resistance/relapse. Here, we report the establishment of a highly sensitive, low cost, in-house nested Reverse Transcription-Polymerase Chain Reaction (RT-PCR)-based assay to detect PhC, and the screening of suspected leukaemia patients for the presence of Bcr-Abl fusion mRNA products. The in-house assay was developed according to previously published literature. Specimens of whole blood (n=98) and bone marrow aspirates (n=4) from suspected CGL and ALL patients referred to Genetech by state hospitals including National Cancer Institute (n=48), and private hospitals, were screened. Total human cellular RNA extracts of specimens were subjected to nested RT-PCR. The negative controls were water and RNA extracts of normal individuals. The assay yielded either a 456 bp, or a 385 bp PCR product in PhC positive samples, depending on the translocation breakpoints (b3a2 or b2a2, respectively). Seventy three were found to be positive for the PhC while 29 were negative. The 456 bp PCR product was detected in 49 patients and the 385 bp product was observed in 24 specimens. Further, 62 specimens yielded amplification products when both 10 µL and 5 µL volumes of RNA samples were used. In 8 samples, only the 5 µL RNA samples resulted in PCR products indicating PCR inhibition at high concentrations of RNA. In three samples, only the 10 µL RNA samples resulted in PCR products indicating that 5 µL samples had insufficient RNA for detection of PhC. Following drug therapy, re-screening for PhC was performed on three patients diagnosed CGL positive. PCR products were observed in all three cases, however, with much less intensity than the pre-therapy specimens, and in two cases, a product was only observed with 10 µL of RNA, clearly demonstrating that they have responded to therapy.

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