Letter to editor

Quality of Cold Centrifuge Affect on PCR Results

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Dear Sir,

DNA extraction is an important step for PCR test. We are doing DNA extraction by salt-out and Phenol Chloroform method, in which Lymphocytes from whole blood were separated by lysing the red blood cells (RBCs) using a Red blood cell lysis buffer. About 100µl of RBC lysis buffer was added to 500 µl blood sample and mixed by vortexing and centrifuged at 8,000 rpm 4ºC for 10 min. The supernatant was discarded. To the pellet, 200 µl RBC lysis buffer was added, and vortexing and centrifugation steps were repeated two to three times until a clear supernatant and a clean white pellet were obtained. After the final wash, the supernatant was discarded completely, and the pellet was resuspended in 200µl in double distilled water. Pellet was broken with tip, followed by addition of 80µl protienase k buffer + 6µl of 100µg/ml protienase k +10µl 10% SDS + 80µl 5 M NaCl solution. The sample was vortexed to dissolve the pellet completely. Then 200µl double distilled water + 400µl phenol chloroform solution (5:1) was added and mixed by vortexing. The tube was centrifuged at 14,000 *rpm* at 4°C for 30 min, and the aqueous upper layer was transferred to a fresh tube and added two volumes of chilled absolute alcohol and mixes gently and centrifuged it at 14,000 *rpm* at 4°C for 25 min. The supernatant was discarded and 500 µl 70% ethanol was added, and the pellet was tapped gently, followed by centrifugation at 14,000 rpm for 5 min and discarded the supernatant gently. The pellet was air-dried at 40 to 56˚C for 3-4 minutes and then dried pellet was resuspended in 100µl double distilled water and Kept at 56°C for 3-4 hours to dissolve DNA and frozen at -20°C or -70°C in small aliquots for storage.

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We had a new cold centrifuge of PSN Instrumentation Pvt. Ltd, Delhi Company in our laboratory. We started DNA extraction this cold centrifuge and noticed that our results were unsatisfactory concentration of DNA and false bands were produced.

Then we prepared DNA extraction by same method and same reagents on cold centrifuge one other companies M/S Plasto Craft industries (P) Ltd. Mumbai. During centrifugation we observed that cold centrifuge of PSN Instrumentation Pvt. Ltd, Delhi was making more vibration as compared to cold centrifuge of Plasto Craft. Quantification of DNA was very less when it was prepared in PSN cold centrifuge as compared to Plasto Craft cold centrifuge. We performed HLAB-27 typing on 50 samples by conventional allele-specific PCR. The primers used for HLAB-27 were E91s: 5'-GGGTCTCACACCCTCCAGAAT-3'and E136as: 5'-CGGCGGTCCAGGAGCT-3' (1) which produce a 135-bp PCR product from genomic DNA. The primer used for Control primer were β globulin was PCO4: 5'-CAA CTT CAT CCA CGT TCA CC-3' and GH20: 5'-GAA GAG CCA AGG ACA GGT AC-3' (2) which produce a 268-bp PCR product. In result we found that DNA extracted in PSN cold centrifuge gave false positive band in HLAB-27 in 70% cases while DNA extracted by Plasto Craft cold centrifuge did not give any false positive result. HLAB-27 typing was repeated with the kit of BAG Company supplied by M/S Shiva Scientific. It gave equivalent result with PCR which was done with DNA prepared PSN cold centrifuge.

Up till now it was considered that type and amount of sample, its collection, pipettting, quality of distilled water, contamination and quality of reagents, thermal cycle programme and gel electrophoresis affect PCR results (4, 5). But our study suggest that quality of cold centrifuge also affect the quantity of DNA, PCR results and give false positive results and hence cold centrifuge should be of good quality and calibrated.

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Competing Interests

None declared.

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