Table 1: Primers and Probes used for QC-PCR (*)

Primers or probe	Sequence (5´ – 3´)	Localization (EBV B95-8 genome)
QP1	GCCGGTGTGTTCGTATATGG	109462-109482
QP2	bio-CAAAACCTCAGCAAATATATGAG	109652-109675
WT probe	dig-TCTCCCCTTTGGAATGGCCCCTG	109563-109563
IS probe	dig-CTATATGCCTGCTTCCTCCTCCGGCG	

* Stevens et al, 1999 [10]

Amplification reactions were carried out at a reaction volume of 50 µl containing PCR reaction buffer (50 Mm kCl, 1.5 Mm $MgCl_2$, 10 Mm Tris (pH 8.2), 200 µM (each) deoxynucleoside triphosphate, and 1U of Taq DNA polymerase (Roch, USA). 25 pmole of Primer QP1. A 25 pmole of antisense primer QP2, 5 µl of DNA elute was added and amplified as follows: Denaturation at 95 °C for 4 min. and subjected to 40 cycles, each cycle consist of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 70 dC for 1 min. Samples maintained at 72 dC for 3 min [10].

Qualitative EBNA-1 PCR:

Standard dilution curve of the wild type (WT) DNA of known copy number was constructed by making serial dilution of WT DNA (106, 105, 104, 103, 102, 101, and 100). Each one of these dilutions was amplified separately. The PCR cocktail preparation performed in a 50 µl volume reaction, Primers and probes are listed in table 1 [10].

The PCR product of each dilution was quantified by ELISA detection method and optical density (O.D) was read at 405 nm.

Standard curve was blotted between O.D. of each PCR product of each dilution and the number of WT copies present in each dilution (Figure 3).

Prescreening method for patients and healthy control samples:

DNA eluted from blood samples of each patient before treatment and controls DNA was amplified as mentioned previously. By comparison of the results obtained to the standard dilution curve, the number of copies of EBV present in each sample was estimated.

Quantitative competitive PCR assay:

Five µl elute of DNA extracted from blood samples of each patient before and after treatment was amplified with IS DNA copies (104, 103, and 102) separately. Primers and probes used as in Table 1.

Quantification of PCR products by enzyme immunoassay (EIA):

EIA with a modified procedure Jacobs, 1996 used Density. Five µl of biotinylated PCR products were added to 50 µl of 1 x hybridization buffer, denatured by 0.2 M NaOH. A 50 pmol/ml WT Digoxigenine (DIG) labeled oligonucleotide probe was added to one of the wells then IS DIG labeled oligonucleotide probe was added to the other well. Antidigoxigenine – conjugated antibodies (75 mU/ml hybridization buffer) added to all wells followed by 100 µl of 2, 2-Azino-di (3-ethyle benzthioazoline sulphonate 6) diammonium salt (ABTS) substrate (Roch. USA). The color intensity was measured at 405 nm and the runs included positive control PCR product, where as distilled water (D.W) was used as negative control [11].