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Letters

HER-2/*neu* Amplification in Human Breast Cancer: Southern or Slot Blotting For Amplification Analysis?

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SINCE THE paper by Slamon *et al.* [1], HER-2/*neu* amplification has been found in a significant number of human breast cancers with a frequency ranging from 10 to 31% (reviewed in [2]). Southern blotting was the most frequently used method whereas slot blotting was used more rarely. The aim of the present study was to compare the ability of each method to evaluate the HER-2/*neu* amplification with the final goal of validating slot blotting for routine use in a hospital molecular biology laboratory.

HER-2/*neu* amplification analysis was realised in 201 histologically proven breast cancers. The reference DNA was obtained from healthy donor leucocytes. Genomic DNA was extracted by Dykes' modified method [3]. In the purification step, Triton X-100 was substituted for sodium dodecyl sulphate (SDS) as SDS interferes with the DNA binding during the subsequent chromatographic stages (Qiagen). For Southern blotting, *Eco*RI restricted DNA samples (10 μg) were blotted onto a Hybond N+ membrane (Amersham) using the Vacugene system (Pharmacia LKB). For slot blotting, three DNA samples (10 μg) were blotted onto a nylon membrane (Bioprobe System). Three cDNA probes were used: HER-2/*neu*, a 1.03 kb *Eco*RI fragment; p53, a 1.40 kb *Nco*I-*Bam*HI fragment; and β-globin: a 1.90 kb *Bam*HI fragment. Each cDNA probe was ^{32}P-labelled by nick translation (specific activity: 3–5 × 10^7 cpm/μg). For slot blotting, the HER-2/*neu* probe was a 24 mer ^{32}P-labelled oligonucleotide (Cliniscience). Hybridisation was performed at 42°C (cDNA) or 50°C (oligonucleotide probe). The filters were autoradiographied using X-OMAT film (Kodak) exposed with intensifying screen at −70°C for 24–72 h. Hybridisation signals were quantified by laser densitometry (Ultroscan XL, Pharmacia LKB).

Southern blotting, considered as the reference technique, was used in 151 cases. The membranes were hybridised simultaneously with HER-2/*neu* and two control probes used to correct the hybridisation signal for the amount of DNA blotted: β-globin and p53. The amplification level was evaluated by the ratio of the corrected signal for the tumour DNA to the corrected signal for leucocyte DNA. There were 28 amplified tumours (18.5%) with a ratio equal to or higher than 2.0. Slot blotting was applied to 151 samples. The membranes were hybridised successively with the three probes. The degree of amplification was calculated by the ratio of the mean intensity (n = 3) of the hybridisation signal for the tumour DNA to the mean intensity (n = 3) of the leucocyte DNA. The signal was corrected as above. Overall, 23 amplified tumours (15.2%) were detected. The 101 DNA analysed by both techniques, using the same cut-off value, displayed a good agreement between approaches; the specificity was better than the sensitivity (Table 1, P = 0.0001) [4]. However, determination of the slope of a regression curve indicated that the Southern blotting gave relatively higher amplification values.

Table 1. Comparison of the Southern and slot blotting results in 101 samples

	Slot blotting		
	Non-amplified	Amplified	Totals
Southern blotting			
Non-amplified	81	3	84
Amplified	4	13	17
Totals	85	16	101

No statistically significant difference between the two techniques (χ^2-test, P = 0.0001).
Specificity = 0.96; sensitivity = 0.77; negative predictive value = 0.95; positive predictive value = 0.81.

Therefore, it appears that the results obtained by each method could be used for the assessment of the HER-2/*neu* amplification, so long as the results are expressed as amplified vs. unamplified; more caution should be taken when the actual amplification values are evaluated. This is important to consider as the slot blotting is technically simpler and easier to implement for routine amplification analysis in a hospital medical laboratory.

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