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Technical Note

The Performance of a Commercial Radioligand Binding Assay for the Epidermal Growth Factor Receptor is Comparable to the EORTC Standard Assay

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THE PRESENCE of epidermal growth factor receptors (EGF-R) was recognised to be indicative of poor prognosis in human breast cancer, although its significance as a prognostic marker for response to therapy and progression of tumours has remained a matter of controversy [1–4]. A variety of methods were developed to quantify levels of EGF-R expression, in particular binding assays with radiolabelled or biotinylated ligands, immunoenzymatic assays, immunohistochemistry, and mRNA analysis. The majority of studies were based on radioligand binding assays, but even among these, a great divergence of results was apparent. These variations were, at least in part, caused by the lack of standardised methods for sample processing, radiolabel preparation, receptor analysis, and measurement of reference parameters, such as protein concentration.

We performed a series of experiments at a quality control laboratory of the European Organization for Research and Treatment of Cancer (EORTC) Receptor Study Group (University Hospital Sint Radboud, Department of Experimental and Chemical Endocrinology, Nijmegen, The Netherlands) to assess the performance of a commercially available, multiple point radioligand binding assay for EGF-R (EGF-Receptor "Scatchard" Assay, ViennaLab, Vienna, Austria) compared with the method that has recently been recommended by the Receptor Study Group as the common methodology for assaying EGF-R [5–7]. The study was designed to examine whether these currently accepted common standards are met by a ready-to-use kit that is available for any non-specialist laboratory.

The EORTC assay for EGF-R was performed as described in detail elsewhere [5, 7]. The ViennaLab "EGF-Receptor Scatchard Assay" was used according to the protocol provided. The two assays have certain characteristics in common (e.g. the use of enzymatically labelled [^{125}I]-EGF at eight increasing concentrations), but differ in several important respects, such as incubation conditions and the type of reagents used for the separation of bound and unbound tracer. Receptor values from both assays were calculated by Scatchard analysis using a curve-fit computer program ("Receptor") designed by H.A. Ross (University Hospital Nijmegen, The Netherlands). EGF-R analyses, resulting in interpretable Scatchard plots, were classified as positive.

Initially, the within (intra-) and between (inter-) assay reproducibilities of both methods were analysed using human placenta membrane preparations [8]. The data obtained, expressed as coefficients of variation (C.V.) in Table 1, confirmed both assays to be highly reproducible and suitable for routine applications. In a subsequent series of tests, one hundred primary breast carcinomas were processed in accordance with EORTC recommendations for steroid hormone receptor assays [9]. Membrane suspensions were obtained as previously described [7], and analysed for protein content (Membrane Protein Assay, ViennaLab; range of results: 0.32–2.44 mg/ml) and for EGF-R, in parallel with both the EORTC and the ViennaLab assay (Table 1). Slightly more than half of the tumours were found to be EGF-R positive (EORTC: 56%, ViennaLab: 58%). With the exception of one sample (>1000 fmol/mg), EGF-R values were between 1 and 200 fmol/mg of membrane protein. By applying the Spearman rank order correlation test, data were shown to be in excellent agreement ($r = 0.9084$, $P < 10^{-6}$). Upon classification of results into "EGF-R-positive" and "EGF-R-negative", accordance of data was found in 92 of 100 samples. Both assays exhibited very low non-specific tracer binding (EORTC: 2.5%, ViennaLab: 1.5%, when expressed as the ratio of non-specifically bound tracer to free tracer), which is a major prerequisite for a sufficiently high sensitivity.

Based on these results, it can be concluded that both assays generate comparable data, and that it is possible to utilise a commercially available assay for routine measurement of EGF-R in human tumour specimens according to EORTC standards.

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Table 1. Comparison of EORTC and ViennaLab assay

	EORTC assay	ViennaLab assay
Intra-assay reproducibility*	$n = 5$ (sample A)	$n = 5$ (sample A)
Mean EGF-R value	30.2 fmol/ml	29.0 fmol/ml
S.D.	2.1 fmol/ml	1.3 fmol/ml
C.V.	7.1%	4.4%
Inter-assay reproducibility*	$n = 9$ (sample B)	$n = 9$ (sample C)
Mean EGF-R value	98.6 fmol/ml	571 fmol/ml
S.D.	8.1 fmol/ml	32 fmol/ml
C.V.	8.2%	5.7%
EGF-R values†		
Positives	$n = 56$	$n = 58$
Median value	18.2 fmol/mg	12.9 fmol/mg
Mean value	49.6 fmol/mg	45.5 fmol/mg
Range	1.6–1036 fmol/mg	1.6–1084 fmol/mg
Correlation of results†		
EORTC +/VL + ($n = 53$)	Accordance of EGF-R positive/negative in 92% of samples	
EORTC +/VL − ($n = 3$)		
EORTC −/VL + ($n = 5$)	Spearman rank order correlation of overall positives ($n = 53$):	
EORTC −/VL − ($n = 39$)	$r = 0.9084$, $P < 0.000001$	
Non-specific binding† (median bound/free ratio)	2.5%	1.5%

*The intra (within) and inter (between) assay reproducibilities of both methods were determined by a single person analysing human placenta membrane preparations for the indicated number of repeats (n) in one single (intra-assay) or in multiple (inter-assay) sets of assays. (S.D., standard deviation; C.V., coefficient of variation); †A total of 100 primary breast carcinomas were analysed in parallel using both assays, and EGF-R values were calculated by Scatchard analysis. Data were compared using basic and non-parametric statistics. (Spearman rank order test: r, correlation coefficient; P, significance level.)

For a final decision on the value of EGF-R as a prognostic parameter, clinical data from a sufficiently large number of patients will have to be compared to EGF-R levels obtained under standardised assay conditions. The present study opens the possibility for oncologists and routine diagnostic laboratories to acquire EGF-R data by means of a commercially available assay according to European standards set by the EORTC.

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