Single-day FISH procedure for paraffin-embedded tissue sections using a microwave oven

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The fluorescence in situ hybridization (FISH) technique is a sensitive method for detecting gene amplification in tumor cells. However, FISH on formalin-fixed and paraffin-embedded tissue sections is time-consuming and labor-intensive (1). Tissue processing and cross-linking fixatives such as formalin may prevent the probe from accessing its target sequence, prolonging the FISH processing time for fixed tissues compared to cells in suspension or frozen material (2,3). For example, the standard FISH procedure for fixed tissue takes about 2 days and contains several acid/chaotrope-based pretreatment steps and an overnight hybridization (www.vysis.com/PretreatingParaffinSpecimens_32217.asp). Despite its sensitivity and specificity, FISH on paraffin sections is therefore not the method of choice in many clinical and research laboratories. Here we propose a simplified and rapid FISH protocol for fixed tissues using microwave irradiation.

Using the commercially available probe mixture of \textit{HER-2 LSP} (locus-specific probe) and \textit{CEP17} (centromere enumeration probe for chromosome 17, α-satellite DNA; Vysis/Abbott, Downers Grove, IL, USA), FISH was conducted on 4 μm thick formalin-fixed, paraffin-embedded tissue sections from 9 breast cancer and 7 endometrial cancer cases. Normal human lymphocytes and 4 breast cancer cell lines, all embedded in paraffin, were used as controls for hybridization and scoring efficiency (4). In total, 21 specimens were analyzed. Sections from the same block were treated either with the commonly used FISH procedure developed by Vysis or the new microwave procedure. For each case, an average of 62 (30–173) cells were scored and the mean copy number for each probe, the \textit{HER-2/CEP17} ratio, and the proportion of aneuploidy were compared between the methods (for statistical analysis, see Supplementary Table S1 available online at www.BioTechniques.com).

In this study, a Samsung Microwave 1100W (Model no. MW1080STA, with 10 power levels; Samsung Electronics America, Ridgefield Park, NJ, USA) was used. The microwave settings for other brands of microwaves can be determined by measuring the temperatures in the buffer (pretreatment step) and water (hybridization step). The optimal temperatures are written in parentheses after each irradiation step in Protocol 1. Keep in mind that irradiation time must be optimized to prevent the loss of tissue morphology, but the tissue has to be irradiated long enough for the \textit{LSP} probe to hybridize.

The mean copy number results from the two procedures were highly comparable (see Supplementary Table S1). The microwave FISH procedure performed equally well for both nonamplified and amplified cases as well as controls, with two exceptions. In BT-474 cells, the mean absolute \textit{HER-2} signal per cell ±sd was slightly higher after microwave treatment (34.0 ± 11.0) compared to standard treatment (28.1 ± 10.5), and the difference reached significance ($P = 0.002$). We ascribe the observed difference to the complex nature of the \textit{HER-2} amplification in these cells, rather than to the type of treatment. In BT-474 cells, due to the formation of compact heterogeneous clusters of \textit{HER-2} signals, the identification of individual signals can be difficult and may result in slightly variable numbers of the mean \textit{HER-2} signals per cell in repetitions (4,5). However, the other FISH parameters showed comparable values, and the results from both treatments meet the criteria for \textit{HER-2} amplification status in the BT-474 cells (4,5). The same explanation is applicable to the one cancer case (see Supplementary Table S1, case 14).

Our rapid microwave protocol showed high hybridization efficiency and gave us signals that were as bright (Figure 1, B, D, and F) as the traditional method (Figure 1, A, C, and E). In Figure 1, A and B, adjacent tissue sections from the same breast cancer case are shown. The \textit{HER-2/CEP17} ratios were 4.2 (standard treatment) and 4.5 (microwave) ($P = 0.448$). By

![Figure 1. Comparison of traditional and microwave FISH methods.](Image)

(A and B) Fluorescence in situ hybridization (FISH) on fixed breast cancer and (C and D) endometrial cancer tissue sections from the same case and (E and F) MCF-7 cells treated either with the (A, C, and E) the standard protocol or with (B, D, and F) the microwave protocol. Cells were stained for \textit{HER-2} (red) and \textit{CEP17} (green). Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI).
both methods, this case was classified as HER-2-amplified and polysomic for chromosome 17 ($P = 0.227$) (Supplementary Table S1, case 1). In Figure 1, C and D, adjacent sections from a highly HER-2-amplified endometrial cancer are shown. Analysis revealed a ratio of 15.2 (standard treatment) or 13.9 (microwave) (Supplementary Table S1, case 12). Statistical analysis of combined FISH results confirmed the high concordance between mean HER-2 ($P = 0.945$) and CEP17 ($P = 0.361$) per cell, copy number ratios ($P = 0.777$), and the proportion of polysomic cells ($P = 0.568$) across all 21 cases analyzed by the standard method and by the microwave method. In 5 cases, the microwave procedure was repeated on a second slide with results very close to the first slide, demonstrating high reproducibility (see Supplementary Table S1).

The sections from the paraffin-embedded cell lines and normal human lymphocytes did not require as long a period of microwave irradiation as the primary tumor tissue sections. Both LSP and CEP probes produced bright signals after only two rounds of low-power microwave irradiation (Figure 1F) in contrast to four rounds for tissue sections. The CEP probe hybridized more efficiently than the LSP probe. After only 5 min of microwave hybridization, the centromeric signals could easily be detected and counted even in the tissue sections.

The rapid microwave pretreatment and hybridization technique described here is a simplified protocol with a significantly shortened time for the FISH procedure on fixed material. It allows, for example, clinical information about HER-2 amplification status in breast cancer patients to be obtained in only 1 day. In our laboratory, a single person was able to both process and analyze 3–5 slides within one normal (8 h) working day. Although the processing may be scaled up easily using the proper equipment, the microscopic analysis is still done manually and is thus the time-limiting step. This protocol eliminates the use of large quantities of potentially dangerous chemicals (e.g., formamide, sodium thiocyanate, and formalin).

The use of microwave irradiation in different in situ hybridization (ISH) applications has been previously reported. Microwave irradiation has been applied during pretreatment and/or for hybridization (2,6–8). It has been shown to markedly shorten the ISH protocols and enhance the hybridization efficiency of weak signals, especially on metaphase spreads. Microwave-based FISH on paraffin-embedded tissue sections has been described in three reports (1,9,10). Two of them use the microwave during the pretreatment step of FISH on fixed prostate tissues (1) and breast tumors (10). Kitayama et al. (9) has described a procedure using microwave during both pretreatment and hybridization steps, reducing the hybridization time from 14–18 h to 3–6 h, but only for chromosome enumeration FISH probes on fixed gastric tissues.

By applying our rapid microwave-based pretreatment/hybridization FISH protocol to breast and endometrial tissue sections in paraffin, we achieved an efficient hybridization independent of tissue type. In addition to HER-2 and CEP17, we successfully performed MYCE/CEP8 FISH on two breast cancer cases (data not shown) and confirmed the suitability of the microwave-based method for hybridization with different LSP probes.

We find the method described very promising and will continue to use it in a larger study. In breast cancer, the HER-2 status detected by FISH is considered to be a more accurate predictor of response to Herceptin than that detected by immunohistochemistry (IHC) (11) but the procedure could be cumbersome (1). IHC, on the other hand, is a fast and easily automated method. With the rapid microwave-based pretreatment/hybridization technique, FISH may become an accessible method in every clinical laboratory and a truly efficient alternative to IHC.

**COMPETING INTERESTS STATEMENT**

The authors declare no competing interests.

**REFERENCES**


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