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ENZYMATIC METALLOGRAPHY: A SIMPLE NEW STAINING METHOD

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The enzymatic conversion of an organic substrate to an intensely colored, insoluble reaction product is widely used as a detection method in immunohistochemistry [1], and has also been used for in situ hybridization detection [2]. Although widely used, it is not ideal for all applications. Diffusion of the products may limit resolution, and the continuous nature of the staining may obscure the underlying cellular structure. Contrast with other cellular stains may be less than desired, an issue which has been addressed by the recent development of nickel-based enhancement reagents that bind to the diaminobenzidine reaction product to give a darker signal [3]. Some applications also require higher sensitivity than is possible with direct enzymatic staining; this is usually achieved through an amplification method such as the polymerase chain reaction [4] or catalytic deposition of haptenated substrates such as biotinylated tyramides [5]. However, this adds substantially to the length and complexity of the procedure, and may also be subject to diffusion of the amplified products.

We have found that horseradish peroxidase-conjugated probes can, in the presence of an appropriate metal source and activating agents, selectively deposit metal to give a black, highly localized stain. The results shown in Figures 1-4 show comparisons of the new metallographic substrate and conventional DAB development in human breast carcinoma. Paraffin sections were deparaffinized in xylene, hydrated and subjected to HIER (antigen retrieval) using steam heat for 30 minutes. After endogenous peroxidase was blocked with a 3% H_2O_2 solution for 5 minutes, the sections were incubated with the primary antibody for 30 minutes, followed by incubation with Envison detection kit (Dako Corp) for 30 minutes. Controls (Figures 1 and 3) were stained with DAB chromogen for 5 min and counterstained with hematoxylin for 2 minutes; experimental sections (Figures 2 and 4) were treated with the metallographic substrate for 7-8 minutes and counterstained with methyl green. The punctate nature of the staining compared with DAB and very low degree of background binding is clearly apparent. The method was also found to be highly sensitive for in situ hybridization detection (Figure 5), and yielded considerably darker staining than found with DAB in parallel immunoblots (Figure 6).

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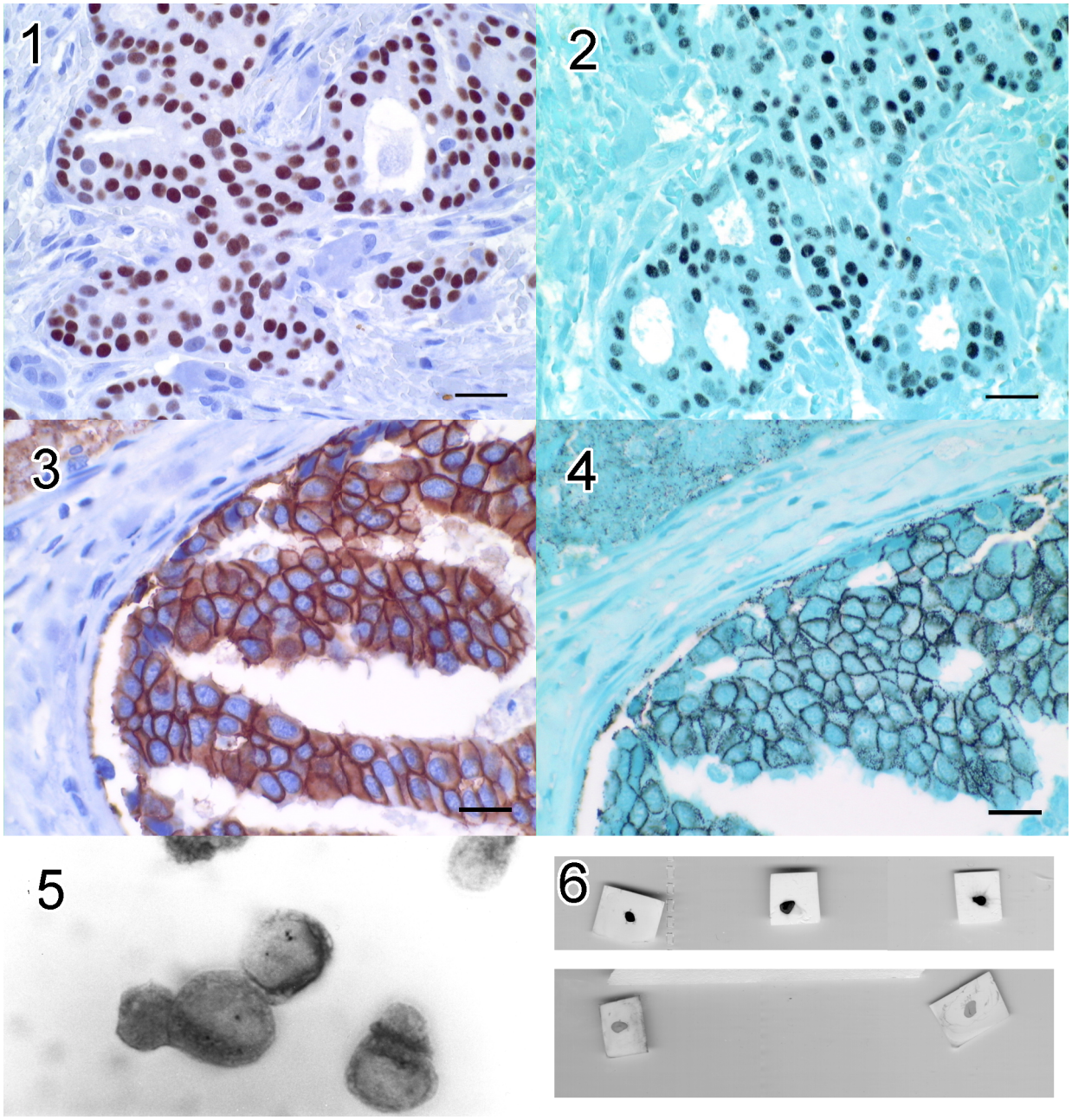

Figures 1 and 2: Infiltrating duct carcinoma of the breast stained for Progesterone receptor (monoclonal antibody PR-88), followed by (1) DAB or (2) enzyme metallography (bar = 50 µ).

Figures 3 and 4: Intraductal comedocarcinoma of the breast, stained for Her 2/neu using polyclonal c-erb-B2 primary antibody, followed by (3) DAB or (4) enzyme metallography (bar = 25 µ).

Figure 5: In situ hybridization detection of Her-2/neu in a moderately amplified control cell line (4-6 copies); each spot corresponds to an occurrence of the gene. Biotinylated probe, detected with streptavidin – horseradish peroxidase, and metal deposition mixture (x 1,000 magnification).

Figure 6: (top) Immunoblots developed using new metallographic substrate for horseradish peroxidase. 2 micrograms of HRP were deposited onto nitrocellulose, blocked with 4% BSA, then treated with metallographic substrate; (bottom) same target developed with standard DAB.

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