

# DAB and the Importance of Separating Waste

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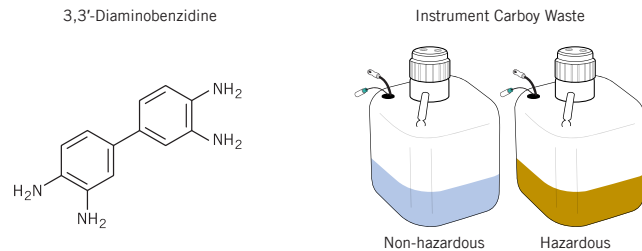
3,3'-Diaminobenzidine (DAB) is an organic chemical compound that is widely used in immunohistochemical (IHC) staining for its ability to produce an insoluble brown pigment in the presence of peroxidase enzyme. For decades, the rich brown stain produced by DAB has made it the chromogen of choice for IHC stains. However, precautions must be taken due to the fact that DAB is a known mutagen and potential carcinogen.<sup>3</sup> The risks associated with DAB usage and disposal have been a matter of discussion and regulation for decades.

It is well established that benzidine and its derivatives such as DAB induce DNA damage and mutagenesis in controlled experiments.<sup>4</sup> In 1977, the Occupational Safety and Health Administration (OSHA) also classified DAB as a Category I Carcinogen. To meet this criterion, evidence for its carcinogenicity had to be based on human data and be replicated in tests on at least one mammalian species.<sup>2</sup> Studies have shown that benzidine is rapidly taken up by the kidneys, lungs, and liver, and it is theorized that its metabolism in the body creates reactive derivatives that contribute to tumor formation.<sup>2</sup>

Since DAB is considered a hazardous material, disposal of DAB-contaminated waste is subject to environmental, health and safety regulations. Regulations are set on both the federal and local level, and so may vary from county to county. Generally, laboratories require DAB-contaminated waste to be hauled away and disposed of by special services.

Automated staining instruments will produce a higher volume of waste than manual staining, exponentially increasing waste generation and disposal for laboratories that choose to automate. Fortunately, the majority of IHC staining waste is frequently considered non-hazardous by local regulations. However, DAB contamination has the potential to render all generated waste hazardous, increasing professional disposal costs. Therefore, it is advantageous for instruments to separate DAB waste from other nonhazardous waste so that laboratories will only be required to pay to professionally dispose of what is absolutely necessary. Waste separation is a factor that laboratories should consider when comparing automated staining platforms.

## DAB Waste Separation on Automated Platforms



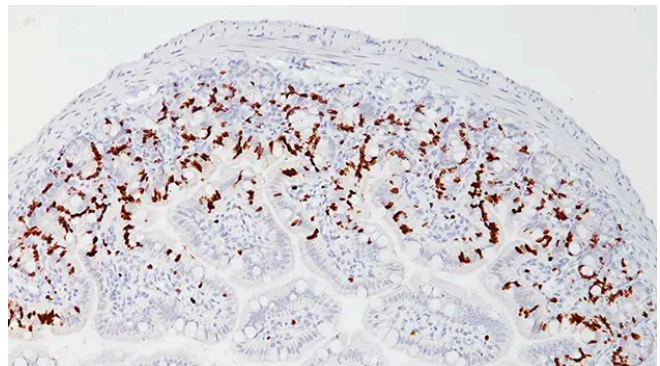
It is for these reasons that all of Biocare Medical's IHC instruments automatically separate hazardous from nonhazardous waste. This environmentally-conscious choice helps laboratories save both time and money spent on waste disposal.

Of course, laboratory staff should always consult OSHA, federal, state, or local regulations for disposal of any toxic substances. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.

## Antibodies Samples Stained Utilizing DAB HRP



Colon cancer stained with Pan Cytokeratin [AE1/AE3] antibody



Bromodeoxyuridine positive mouse intestine stained with anti-BrdU antibody

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2. Frohliger, John O.; Kotsko, Nancy (1982). Ion exchange chromatographic separation of benzidine, 3,3 Dichlorobenzidine and 3,3 Diaminobenzidine. *Journal of Environmental Science and Health . Part A: Environmental Science and Engineering*, 17(5), 675-681. doi:10.1080/10934528209375070
3. Lunn, George; Sansone, Eric B. (1991). The Safe Disposal of Diaminobenzidine. *Applied Occupational and Environmental Hygiene*, 6(1), 49-53. doi:10.1080/1047322X.1991.10387826
4. Ssu-Ching Chen, Chih-Ming Kao, Mei-Han Huang, Ming-Kuei Shih, Ya-Lei Chen, Shiao-Ping Huang, Tsan-Zon Liu, Assessment of Genotoxicity of Benzidine and Its Structural Analogues to Human Lymphocytes Using Comet Assay, *Toxicological Sciences*, Volume 72, Issue 2, April 2003, Pages 283-288, <https://doi.org/10.1093/toxsci/kg026>
5. Z. You; M.D. Brezzell; S.K. Das; M.C. Espadas-Torre; B.H. Hooberman; J.E. Sinheimer (1993). ortho-Substituent effects on the in vitro and in vivo genotoxicity of benzidine derivatives. , 319(1), 19-30. doi:10.1016/0165-1218(93)90027-b