

## **Steps Used to Replace B-5 Fixative with B-Plus Fixative**

Step 1. Collect and place tissue from normal tonsils in B-Plus fixative and one in B-5 fixative.

Method: Tissue collected from normal tonsils was placed into both B-Plus and B-5 fixative. The B-Plus specimens were allowed to fix for two, four, and six hours. The B-5 specimen was allowed to fix for one hour.

Purpose: To determine optimal fixation time and to compare preservation of nuclear details between both fixatives.

Results: All four specimens were processed routinely after the initial fixation in B-Plus and B-5 fixative. Sections were cut and stained with Hematoxylin & Eosin. The B-5 specimens received deenzkerization treatment to remove mercury pigment. Unmarked slides were given to our pathologists for review. The first slide that was rejected was the B-5 slide. The pathologists found that the nuclear details were preserved best in the B-Plus specimens. The best results were obtained in the specimens fixed for four and six hours.

Step 2. Collect and place tissue from lymph node specimens in both B-Plus fixative and in B-5 fixative

Method: Tissue collected from lymph node specimens was split and fixed in both B-Plus and B-5 fixative. Specimens were allowed to fix from four to six hours depending on size. The B-5 specimen was allowed to fix for one hour.

Purpose: To determine if B-Plus performs consistently using patient tissue samples.

Results: All specimens were processed routinely after the initial fixation in B-Plus and B-5 fixative. Sections were cut and stained with Hematoxylin & Eosin. The B-5 specimens received deenzkerization treatment to remove mercury pigment. The stained slides were given to our pathologists for review. The pathologists found that the nuclear details were well preserved in the B-Plus specimens and no significant differences between the two fixatives were detected.

Step 3. Sections from the lymph node specimens obtained during step two were stained for the following Immunohistochemical markers;

CD20  
CD3  
CD45

Method: Sections were prepared for Immunohistochemical staining. A steamer containing citrate buffer was used for antigen retrieval. Slides were stained on an automated stainer.

Purpose: To determine the effect of B-Plus on antigen binding sites and IHC stains.

Results: All sections were found to be adequately stained for diagnosis. The B-Plus fixative did not adversely affect IHC staining as the zinc-formalin fixatives previously tested had. Titrations and incubation times did not need adjustment.

Step 4. Collect and place tissue from bone marrow biopsies in B-Plus fixative.

Method: Tissue collected from bone marrow biopsies was placed into B-Plus fixative. The B-Plus specimens were allowed to fix for four hours.

Purpose: To evaluate B-Plus' performance on bone marrow biopsies.

Results: All specimens were decalcified and processed routinely after the initial fixation in B-Plus. Sections were cut and stained with Hematoxylin & Eosin. The stained slides were given to our pathologists for review. The pathologists found the results to be diagnostically acceptable. The B-Plus fixative was found to be as good if not, in some cases, better than B-5 fixative.