

Comparison and Validation Study of Formagel™ and Traditional 10% Neutral Buffered Formalin

Azer Scientific, Inc.

Study conducted by:

Michael LaFriniere, MT (ASCP)

Dr. Virginia Galang, MD

Prepared by:

Ralph Finkbiner

Introduction

Azer Scientific approached an outside pathology laboratory to conduct a comparative study between Formagel™ and standard 10% neutral buffered formalin fixed tissues. This validation study was designed to examine the quality, integrity and preservation of these tissues. The laboratory was informed that Formagel™ was a thickened 10% neutral buffered formalin based fixative developed to offer prevention of container leakage during specimen transportation to and from the laboratory, thereby increasing safety during transportation while maintaining specimen integrity.

Background

Fixation is the first stage in a multistep process to prepare a surgical specimen sample for microscopy or other analysis. Tissue fixation must demonstrate preservation of the integrity and morphology of cells and tissues so they can withstand the harsh conditions of dehydration, clearing, embedding and staining that are performed during routine histological processes. In addition, fixation also helps prevent decomposition, putrefaction, autolysis of tissues and optimizes tissue morphology for proper microscopic evaluation.

The current standard for proper fixation of histological studies, is achieved when a sample of tissue is immersed in a 10% neutral buffered formalin fixative at a minimum volume of 10-20 times greater than the volume of the tissue to be fixed. 10 % neutral buffered formalin has proven, over several years, to successfully diffuse through the tissue to render appropriate fixation processes.

Procedure

- 1) A total of 33 fresh tissue specimens were randomly selected and placed in *PFG-20*, a Formagel™ fixative. Additional samples of the same tissues were also processed in standard 10% neutral buffered formalin liquid fixative as a control or expected result.
- 2) Routine histological sections were processed using the two fixatives followed by microscopic side by side comparison. Various histochemical (6) and immunohistochemical (11) staining procedures were also performed to determine reactivity of the stains to the tissues fixed in Formagel™ fixative.

3) For comparison of the histologic sections, five (5) qualitative criteria were utilized:

- a) Hematoxylin uptake (strength of stain, color, density)
- b) Eosin uptake (strength of stain, color, density)
- c) Cellular details (nuclear/cytoplasmic details, crispness, clarity)
- d) Overall morphology (tissue in its entirety, optimal to render accurate diagnosis)
- e) Background (clean)

The above criteria were evaluated and scored 1-4; score 1 being poor and unacceptable and score 4 being high quality. In the standard histology laboratory scores of 3 or 4 are acceptable for diagnostic purposes. Notation of the background was also noted.

The Immunohistochemistry and histochemical stains were scored 0 (negative) to 4 + depending on the strength of stain. Notation of the background was also noted.

Results

Histologic sections (Hematoxylin/Eosin staining): Based on the first four qualitative criteria and the scoring used to evaluate the fixative properties of *PFG-20*, Formagel™ and 10% neutral buffered formalin, the results demonstrate that these two fixatives are comparable to those routinely fixed in 10% liquid neutral buffered formalin. The majority of the specimens of both fixatives scored 4 on staining uptake, cellular detail, and overall morphology equivalent to routine 10% liquid formalin fixation.

NOTE: One finding noted with the gel (*PFG-20*) fixative was the presence of minimal residual deposits (appearing to be precipitate) that were not identified with 10% liquid formalin. These residual deposits were often noted on the surrounding edges of the tissue and were not superimposed on the actual tissue. The “background” section on the summary worksheet scored the residual deposit findings (1) as minimal/light and (2) as heavy/dark.

Histochemical/Immunohistochemical staining: Based on the criteria used, 0-4+ (depending on the intensity of the stain), the Formagel™ fixed samples demonstrated appropriate staining intensities as expected. Similarly as in the hematoxylin and eosin preparations, a residual on tissue edges was still noted, however, it was of lesser degree and only sporadically noted in a few specimens.

Discussion

The study was requested to validate the quality, integrity and preservation of tissue using Formagel™ (PFG-20) formalin based fixative. Our findings indicate that the gel fixative tissues are comparable with that of 10% liquid formalin on the 33 histologic specimens and the 16 special stains included in this study.