TECHNICAL NOTE

Ikuo Ishiyama,[1] *M.D.*

Rapid Histological Examination of Trace Evidence by Means of Cellophane Tape

REFERENCE: Ishiyama, I., **"Rapid Histological Examination of Trace Evidence by Means of Cellophane Tape,"** *Journal of Forensic Sciences,* JFSCA, Vol. 26, No. 3, July 1981, pp. 570-575.

ABSTRACT: Histological and histochemical examinations of trace evidence were performed by lifting the specimens onto cellophane tape. To ensure adhesion of the samples during the staining, the surface of the tape was coated with 2% agarose after the sampling. These samples were ready for such direct stains as hematoxylin and eosin, benzidine, and periodic acid-Schiff to determine proteins and acid phosphatase. Histological analyses may be performed with the same range of smear preparations.

KEYWORDS: pathology and biology, histology, cellophane tape, histochemistry, biological stains

Cellophane tape is being used for sampling specimens taken in criminal investigations. Recently we investigated the possibility of staining specimens on the cellophane tape with the usual histological methods [*1*]. The results, compared with those from other preparation methods, revealed more decisive information on the morphological features of trace evidence. A brief explanation of this method follows.

Double-sided Scotch® tape is applied to a microscope slide and the specimens are attached after they have been scratched slightly on their surfaces with the tip of fine needle. This procedure allows enough of the specimens to be collected for the investigation of trace evidence. The surface of the microscope slide is then covered with a fresh cellophane paper (nonadhesive!) and pressed evenly with the fingertips so that the specimens are fixed firmly on the tape. This also allows the cellophane tape to adhere firmly to the microscope slide; clouding of the cellophane tape when immersed in water is thus avoided. The nonadhesive cellophane paper is detached gently from the adhesive tape. No disturbance of the specimens on the tape occurs with this procedure. As the fixative qualities of the adhesive diminish in water, the fixed specimens may easily detach from the cellophane tape during staining. This difficulty is eliminated by coating the slide with a gelatin or agarose layer. It seems preferable to use the agarose. The agarose is dissolved in a concentration of 1.5 to 2.0% with water, boiled thoroughly, and maintained at 50°C. The slide is immersed directly in this agarose aliquot for a few seconds, removed, and placed on an ice-cold metal plate. After the

Received for publication 22 Sept. 1980; revised manuscript received 5 Dec. 1980; accepted for publication 10 Dec. 1980.

[1]Professor and chairman, Department of Legal Medicine, Teikyo University School of Medicine, 173-Tokyo, Itabashi, Kaga 2-11-1, Japan.

agarose solidifies, the slide is dried at 50°C and stained by various methods. The illustrated specimens include blood (two years old), seminal fluid (two months old), saliva (one month old), nasal secretions (one month old), and sputa of respiratory diseases (one month old), which are attached to clothes (gauze, cotton, handkerchief, and so on) or paper.

After the staining, excess pigment is thoroughly washed out and the slide is dried immediately at 50°C and mounted with Eukitt (O. Kindler, Germany).

Results

Bloodstains

The bloodstains (Fig. 1) were stained with benzidine. 3-3'Diaminobenzidine (DAB) can be used for this purpose. The fibers are stained a dense blue. Some debris is detached from the fibers and scattered around them. The color development can be preserved for a long time without change.

Seminal Stains

The seminal stains (Figs. 2 and 3) were stained with hematoxylin and eosin. Almost all spermatozoa are located on the fibers, which are stained in spots with eosin.

Fingerprints

The fingerprints on the cellophane tape are stained with Coomassie-Brillant Blue R 250, as is used with gel electrophoresis. Figure 4 represents an example in which the fingertip is pressed directly on the adhesive, while Fig. 5 is taken from latent fingerprints remaining on glassware. The desquamated epidermal cells along the fingerprints are stained brilliantly with this pigment. Some granular particles (aggregations of secreted protein?) are sprinkled densely between them.

Sputa of Respiratory Diseases

Stains of the sputa of various kinds of respiratory diseases can be used for identification and diagnosis. Figures 6 and 7 represent the dried stains of sputa on gauze in a case of acute bronchitis. Giemsa staining reveals the typical accumulation of neutrophil leukocytes,

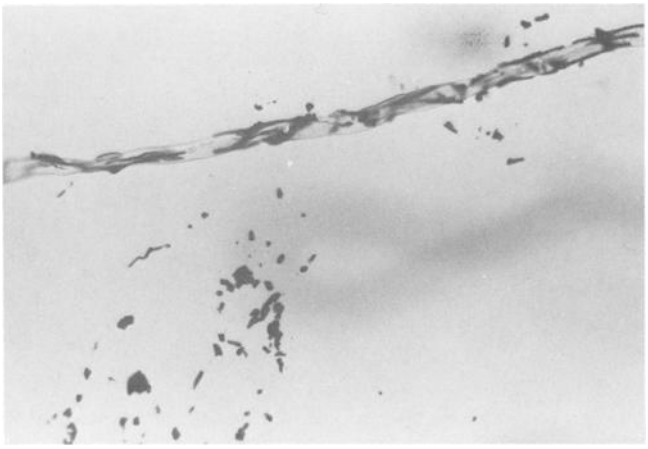

FIG. 1—*Bloodstain, shown with benzidine staining.*

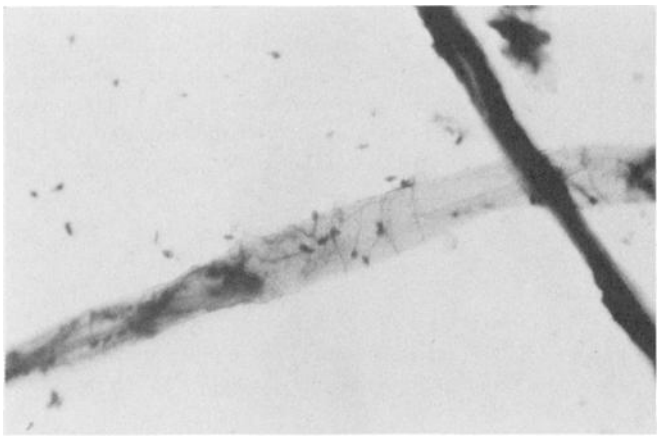

FIG. 2—*Seminal stain, stained with hematoxylin and eosin.*

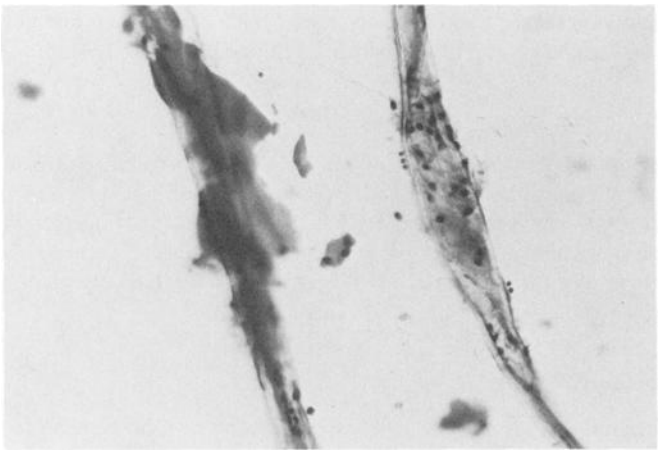

FIG. 3—*Seminal stains adhering to fibers. The left fiber is stained densely with eosin but contains no spermatozoa, while the right one is stained with eosin in spots and contains abundant spermatozoa.*

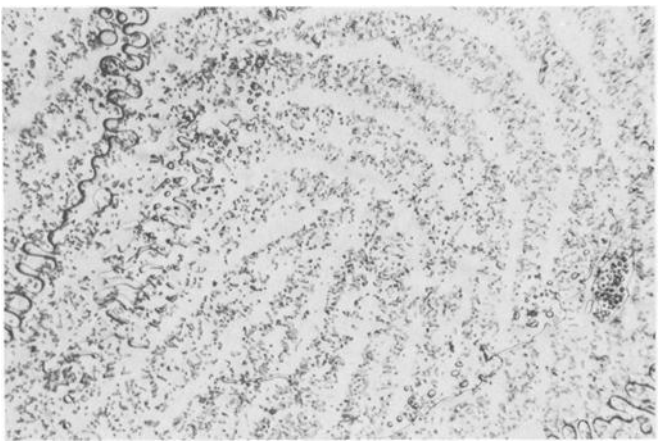

FIG. 4—*Fingerprints on cellophane tape, stained with Brillant Blue. Epidermal linings consisted of desquumated epithelial cells. The fingerprints were left from the fingertip, which was pressed directly onto the surface of the adhesive.*

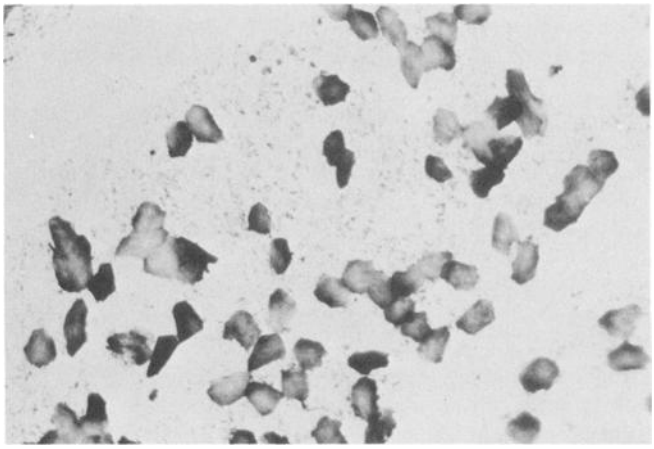

FIG. 5—*Fingerprints on cellophane tape, stained with Brillant Blue. Desquamated cells and granular particles of the epidermal ridge are shown. The sample was lifted from latent fingerprints remaining on glassware.*

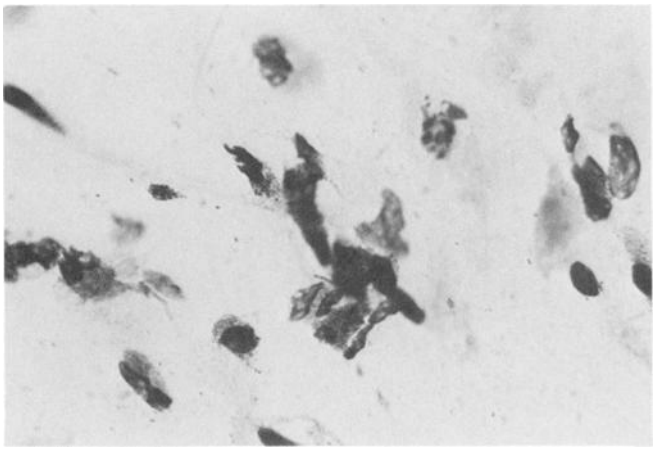

FIG. 6—*Granulocytes (neutrophil) and monocytes with degeneration of nuclei are shown (Giemsa staining).*

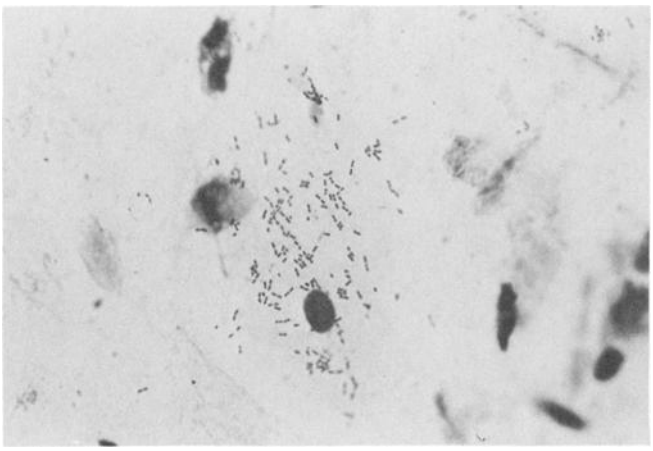

FIG. 7—*Staining of bacteria* (Diplococcus) *on cellophane tape (Giemsa staining).*

macrophages, and the deposition of fibrin with scattered colonies of bacteria of various types. In other cases we found the presence of heart-failure cells (iron-staining) in a case of heart disease and of eosinophil leukocytes in a case of bronchial asthma.

Enzyme Determination

Acid phosphatase activity can be determined with the β-glycerophosphate stain. When enzymes are to be demonstrated any heat treatment should be avoided. Thus, the coating with agarose was performed at 50°C for a short time and stained without drying. Black granules (crystals of lead phosphate) were observed along the fibers (Figs. 8 and 9).

Discussion

It is evident from these findings that the morphological investigation of trace evidence may be executed with the accuracy and simplicity of smear stainings. Magnification as high as ×1000 in oil immersion is possible, so every histological and pathological examination used

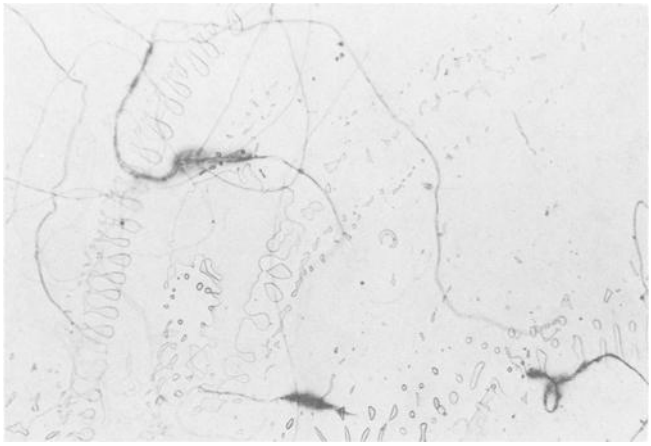

FIG. 8—*Seminal stains, shown using acid phosphatase stained with β-glycerophosphate.*

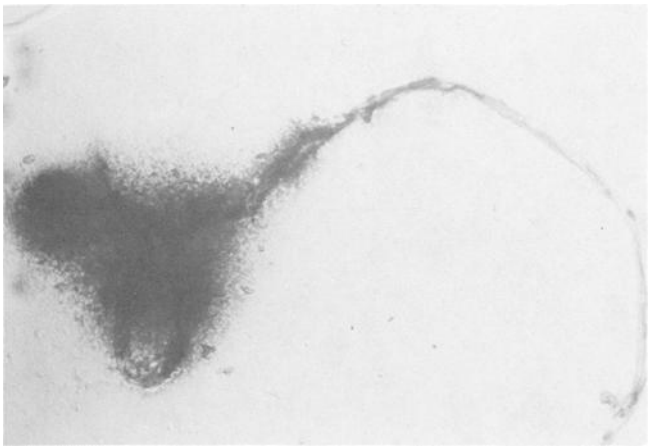

FIG. 9—*Seminal stains, shown using the same method as in Fig. 8. Precipitation of lead phosphate is clearly shown.*

in the routine forensic sciences may be performed without any modification. In this sense, direct staining of specimens on cellophane tape can substitute, on many occasions, for examination by scanning electron microscopy. Furthermore, histochemical techniques (periodic acid-Schiff, enzyme determination; Sudan III cannot be used because of contaminant staining of the cellophane tape) can be achieved in the same way. The other advantages are these: (a) the technique is simple, so that no special equipment is needed; (b) microscopic observations and recordings can be facilitated by color stainings; (c) no destruction of the specimens is necessary and reexaminations can be repeated at the same sites; and (4) other serological and biological (sex determination by X and Y chromatins) tests can be performed with the same technique.

It is suggested that the morphological and biological activities of trace evidence remain in fairly good condition because rapid dehydration prevents postmortem changes. Morphological examination can therefore be regarded as a promising method for ascertaining the actual conditions of trace evidence.

Reference

[1] Ishiyama, I., "Application of Cellophane Tape in Examining Trace Evidence," *Reports of the National Research Institute of Police Sciences*, 1981, in press.

Address requests for reprints or additional information to
I. Ishiyama, M.D.
Department of Legal Medicine
Teikyo University School of Medicine
173-Tokyo, Itabashi, Kaga 2-11-1, Japan