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Evaluation of Two Types of Infectious Mononucleosis Antigen Slides by the Indirect Fluorescent-Antibody Technique

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The efficiency of two types of antigen slides was compared by using the indirect fluorescent-antibody technique. Fifty sera from infectious mononucleosis patients were tested concurrently on the two sets of slides for antibody to Epstein-Barr virus. The indirect fluorescent-antibody serum titer readings from the epoxy slides were either equal to or twofold higher than those from the cover slip slides.

During the past 3 years, our laboratory has been using the indirect fluorescent-antibody (IFA) technique for the diagnosis of infectious mononucleosis (IM) (1). The Epstein-Barr virus (EBV)-IFA procedure, as described by Henle and Henle, has been used routinely (2). The EBV-HR1K cell line (obtained initially from Werner Henle, Children's Hospital, Philadelphia, Pa.) has been the source of antigen, and sera from suspected IM patients have been the sources of antibody. The cover slip slide, the lower slide in Fig. 1, has been the routine antigen slide. The epoxy slide, the upper slide in Fig. 1, has been printed with a durable acetone-resistant hydrophobic epoxy. Each slide has eight circular uncoated 40 mm^2 areas. In this study, we examined 50 sera by the Henle IFA test, by using the cover slip slide and the epoxy slide.

The procedures for preparing the cover slip slides and the IFA method for processing them for reading are as follows. Five cover slips (8 by 30 mm) are taped to a microscope slide (1 by 3 inch). HR1K suspension (0.1 ml) containing 5 $\times 10^5$ cells is placed on each cover slip. An area of about 110 mm^2 of the cover slip is covered by antigen. The cells are air-dried and then fixed with acetone for 10 min. After fixation, 0.1 ml of the test serum is put on each cover slip. The slides are incubated in a moist chamber for 1 hr at 37 C and washed twice with phosphate-buffered saline, *p*H 7.2 After the slides are dried, 0.1 ml of the appropriate dilution of fluorescene-conjugated goat antihuman gamma globulin is added to each cover slip. The procedure of incubation, washing, and drying is repeated again. The cover slips are removed and mounted, cell side down, on a microscope slide with a drop of buffered glycerine (*p*H 7.5) between the cover slip and the slide. The slides are read on a fluorescent-antibody (FA) microscope.

Although the cover slip slide technique yielded dependable measurements, improvements were needed. Preparing the slides was tedious and time-consuming. Larger volumes of reagents were required than we thought were necessary. Occasionally, after a slide was processed for reading, a thin film of unknown origin developed on the slide, making it difficult to read.

We have found the epoxy slide superior to the cover slip slide (Cel-Line Associates, Minotola, N.J.; the epoxy surface material was suggested by Alex Sulzer, Chief of the FA Research Laboratory at the Center for Disease Control). The IFA procedure used with these slides is identical to that used with the cover slip slides except that 0.05 ml of each patient's serum and fluorescene-labeled antihuman globulin is required. Each of the circular uncoated areas requires half the volume of test materials required for the cover slip slide. No laboratory time is needed to prepare the initial slides because they are ready to use as they come from the manufacturer. Since no film develops on the slides during processing, less time is involved in reading them and more accurate readings are obtained. It costs 5 cents to titrate each serum on the epoxy slide and 15 cents on the cover slip slide.

To compare the efficiency of these two types

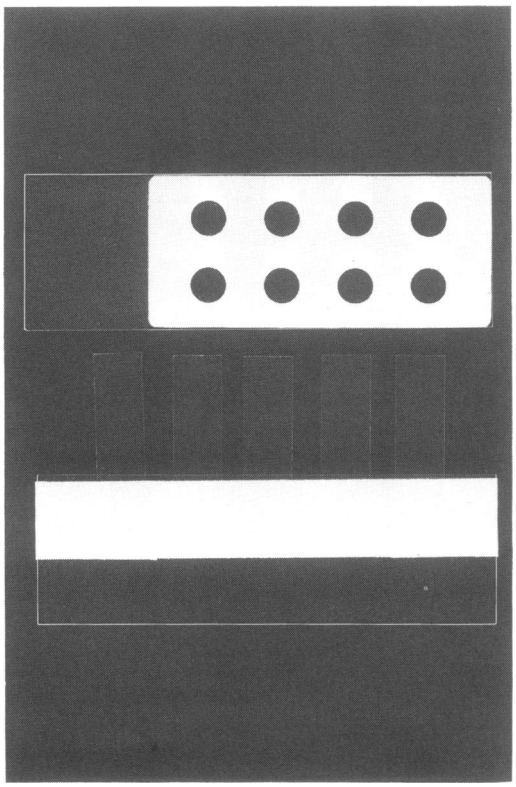

FIG. 1. *Epoxy slide (upper) and cover slip slide (lower).*

of slides, the antigen slides were prepared simultaneously from the same HR1K cell suspension; 5×10^5 cells were placed on a cover slip or a circular area. Fifty routine IM sera were tested concurrently on each of the two sets of slides for antibody to EBV by the IFA procedure. The same laboratorian read all of the slides on one Zeiss FA microscope.

The results in Table 1 show that serum titer readings obtained with the epoxy slides were usually equal to or twofold greater than those obtained with the cover slip slides. Twenty-one of the sera had the same titers on both types of slides. The epoxy slide IFA readings on 28 of the sera showed a twofold or greater increase in titer. One serum had a twofold lower titer. The tendency toward higher titer with the epoxy slides is probably because the epoxy slides receive more reagent per millimeter^2. The epoxy slides receive 0.05 ml per 40 mm^2, whereas the cover slip slides receive 0.1 ml per 110 mm^2.

TABLE 1. *Number of sera showing each fluorescent-antibody serum titer to Epstein-Barr virus with two types of slides*

Cover slip titer	Epoxy[a] titer	<10	10	50	100	200	400	800
<10		7[b]	1					
10			4	6				
50				2	4			
100					1	4	1	
200						3	7	
400						1	4	5

[a] End point dilution factor.

[b] Underlined figures = number of sera giving equal titer with both types of slides.

We find that the epoxy slide also gives clear, dependable serum titer readings by the FA technique when cytomegalovirus and other herpes-like viruses are used as antigens.

We recommend epoxy slides over cover slip slides for the following reasons. (i) They come ready to use from the manufacturer, so no laboratory man-hours are used to prepare the initial slides. (ii) Approximately half the volume of serum and fluorescence-labeled antihuman globulin are required. (iii) Less time is needed to prepare the slides for reading. (iv) No film develops on the slides, so more accurate readings are obtained. (v) Serum titers are equal to or twofold higher than those obtained with cover slip slides. (vi) The epoxy slides cost less than the cover slip slides.

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