Rapid Fluorescent-Antibody Staining Technique

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The rapid fluorescent-antibody staining technique described by Kellogg and Deacon for staining Neisseria gonorrhoeae and Treponema pallidum was applied to fluorescent-antibody tests for group A streptococci and enteropathogenic Escherichia coli. Results obtained with this staining technique were compared with results using the conventional staining procedure; excellent correlation was obtained. Considerable time and materials were saved by using the rapid method; it was also found completely satisfactory.

The standard, generally accepted staining procedure for the fluorescent-antibody method of detection of group A streptococci and enteropathogenic Escherichia coli involves staining the fixed preparation with conjugated antiserum in a moist chamber at room temperature for 15 to 30 min, and then washing the preparations for 10 min in buffered saline. This staining process (exclusive of preparation of the specimen, preparation of film, and mounting) requires 25 to 40 min.

Kellogg and Deacon (2) described a rapid staining schedule for the fluorescent-antibody test for Neisseria gonorrhoeae and Treponema pallidum, in which the reaction between the antigen and conjugated antiserum is carried out at a higher temperature for a shorter period of time. The conjugate is dried on the film preparation at 45 C, requiring about 30 sec to 1 min. The slide is rinsed under running tap water, and then is mounted as usual. Total staining time is less than 2 min.

Since this rapid staining technique offered definite advantages over the longer procedure, it seemed desirable to attempt application of this method to fluorescent-antibody tests for other microorganisms. A study was made, therefore, to evaluate the rapid staining procedure as used in the detection of group A streptococci and enteropathogenic Escherichia coli serogroups.

MATERIALS AND METHODS

Streptococci. A series of 600 unselected routine throat cultures, submitted to this laboratory for examination for the presence of group A streptococci, was prepared by adding about 1 ml of Trypticase Soy Broth to the infusion agar slant inoculated by the physician with clinical material. The slants, with broth, were incubated at 37 C for about 2 hr. The broth was then poured off, sedimented, and washed in buffered saline. Duplicate preparations, consisting of two film preparations on each of two slides, were made from each specimen. Each was fixed in 95% ethyl alcohol for 1 min and then air-dried.

One slide of each pair was stained with the standard procedure, as described by Cherry, Goldman, and Corski (1). One drop of appropriately diluted group A Streptococcus conjugate (Sylvania) was evenly spread on one of the preparations; appropriately diluted conjugated normal rabbit serum (Sylvania) was spread on the preparation on the other end of the same slide. The slide was then placed in a moist chamber at room temperature and allowed to react for 30 min. It was rinsed for 10 min in buffered saline (pH 7.2), then mounted in buffered glycerol, and covered with a number I square cover slip. All preparations were examined with a Leitz Ortholux fluorescence microscope fitted with a 95X fluorite oil immersion objective with diaphragm, a monocular 10X ocular lens, and an immersion dark-field condenser. The light source was an Osram HBO 200 mercury arc burner. A 3-mm BG 12 exciter filter and a 2.5-mm OG 1 barrier filter were used.

The second slide of each pair was stained by the Kellogg and Deacon rapid technique (2). After fixation, one drop of conjugate was spread on each preparation, and the slides were placed in a small, slotted, open-bottom metal slide rack. The rack was placed in an incubator (45 C) so that the slides were in a vertical position. Only a thin layer of conjugate was left on the preparation. The slides were left in the incubator for 30 sec to 1 min, by which time the material appeared to be dry or nearly dry. The slides were left in the rack, and the rack was held under the water faucet. The water was turned on to permit the stream to flow gently over the slides, from edge to edge, so that no cross-contamination could occur from one slide to another. The preparations were blotted, mounted in buffered glycerol, and examined, as in the longer procedure.

E. coli. A similar comparison of duplicate slide preparations stained by the two methods was made of a series of 251 unselected fecal specimens submitted for examination for the presence of enteropathogenic E. coli serogroups.
The specimens were prepared by the method described by Martin and O'Brien (3). Fecal specimens in glycerol were streaked on blood plates, and a loopful of growth from the blood plate was suspended in saline. The saline suspension was used to prepare duplicate sets of slides. One slide was stained by the method obtained from the New York State Department of Health Laboratory, based on procedures used at the National Communicable Disease Center. The film preparations were heat-fixed; one drop of conjugate was evenly spread on each preparation. Difco Bacto-FA E. coli poly A, poly B, and monovalent 0112:B11 conjugated antisera were used on all specimens. When fluorescent bacilli were found in one of the polyvalent conjugates, Difco monovalent antisera constituting that pool were used. (Poly A contains 055:B5, 0111:B4, 0127:B8, and 026:B6; poly B contains 0119:B14, 0125:B15, 0126:B16, 086:B7, 0128:B12, and 0124:B17.) The preparations were stained for 15 min at room temperature in moist chambers, rinsed in saline, and then rinsed in buffered saline for 10 min. They were mounted in buffered glycerol.

After fixation, the duplicate slide preparations were stained with E. coli conjugates according to the same schedule described for streptococci.

**RESULTS**

Streptococci. Complete agreement was obtained in the final results of examinations of the duplicate preparations of 600 specimens examined for group A streptococci; they were found in 160, not found in 440.

In all instances in which there was any question about the results of the fluorescent-antibody test, the test was repeated on the original material, or on growth from a blood plate inoculated with the original material, or both. Examples of findings considered questionable are low degree of fluorescence, not entirely typical morphology, and very few fluorescing microorganisms.

By use of the long staining procedure on this series of specimens, 154 contained group A streptococci upon original examination. It was deemed necessary to repeat the test on 34 specimens. Group A streptococci were found in 6 of the 34 repeats.

By using the rapid staining procedure on the same series of specimens, group A streptococci were found in 159 on the original examination. Only three had to be repeated. One of the three contained group A streptococci.

E. coli. Equally good correlation was found in the duplicate tests for enteropathogenic E. coli, with the two staining procedures on 251 fecal specimens.

Complete agreement was obtained with 243 specimens; enteropathogenic serogroups (one or more per specimen) were found in 47, not found in 196.

Eight specimens produced different results by the two methods, but all of these concerned serogroups 0112:B11 and 0124:B17 (neither of which is currently being reported by this laboratory unless culturally isolated). Both of these serogroups are known to produce troublesome cross-reactions with other gram-negative bacilli.

Neither the 0112:B11 serogroup nor the 0124:B17 serogroup was found in seven of the preparations processed by the rapid method, but one or the other serogroup was found in the corresponding seven preparations processed by the longer procedure. The eighth specimen contained fluorescing bacilli in very small numbers in the rapidly stained slide, but not the slide stained by the standard method.

**DISCUSSION**

Since the results of tests with the staining techniques were in complete agreement (excluding E. coli serogroups 0112:B11 and 0124:B17), use of the rapid staining procedure results in no loss of accuracy. In addition, the rapid procedure has definite advantages over the standard procedure.

(i) The most important advantage of the rapid procedure is the obvious saving of total time consumed in the process. It also makes it possible to start microscopic examinations earlier in the day, thus making results available earlier and permitting examination of a larger number of specimens during working hours.

(ii) The results obtained are more decisive. The fluorescence of the microorganisms is as bright or brighter with the shortened staining period compared with the longer method. The amount of background fluorescence is reduced, producing cleaner preparations. The need for repeating tests is greatly reduced, since questionable results are less frequently encountered.

(iii) A smaller working area is required, since the slides do not have to be spread out on a workbench surface and covered with bulky moist chambers.

(iv) The rapid method does not require the use of large quantities of buffered saline used in the conventional washing procedure.

(v) There is no chance of cross-contamination from one preparation to another due to wash-off, since the slides are rinsed in running tap water. Comparison of duplicate film preparations stained by the two methods failed to show any appreciable loss of material caused by washing in running water.

In summary, the rapid staining method is as accurate as the standard method, quicker, easier, more efficient, and more economical for the tests studied. It seems probable that this method could be applied to other fluorescent-antibody tests with equal success.
LITERATURE CITED