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Automated Instrument for the Fluorescent Treponemal Antibody-Absorption Test and Other Immunofluorescence Tests

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An automated diagnostic test instrument and its development program are described. The instrument automates the fluorescent treponemal antibody-absorption test for syphilis to the extent that only 4 hr of technician time is required to conduct approximately 200 tests daily. Evaluation to date suggests its efficacy. In addition, preliminary studies indicate the feasibility of detecting antibodies to *Toxoplasma gondii*, *Plasmodium malariae*, and nucleoprotein (antinuclear factor). The instrument would seem to have broad application for routine and research immunofluorescence testing. Two elements comprise the instrument: a slide processor and a microscope attachment. The slide processor is an electro-pneumatically actuated device which automatically feeds special laboratory slides, on which antigen or other reagents are prefixed, through a series of operations which provide reagent application, incubation, washing, drying, and stacking of the finished slides for readout. The instrument provides flexibility in that incubation time and temperature as well as point, sequence, and duration of reagent application can be varied to accommodate a variety of immunofluorescence techniques. The microscope attachment can be fitted to all conventional dark-field fluorescence microscopes and makes possible the reading of three to six slides per minute. The reacted slides from the processor are injected sequentially onto the stage of the microscope by movement of a lever. As injected, slides are automatically in visual focus; fine focus is occasionally required. Scanning of the reacted field is accomplished by means of the normal microscope controls. A buffered glycerol coupling is maintained between the dark-field condenser substage lens and the slide cover glass by means of a pushbutton-actuated feed system.

Aerojet-General Corp., Chemical & Biological Division, under contract to Department of the Army, Rapid Warning Office, Fort Detrick, Frederick, Md., developed a number of immunofluorescence systems for the specific detection of biological agents in air samples. Certain techniques developed during that time appeared applicable to the automation of current, manually conducted immunofluorescent diagnostic tests. A survey of such tests was made which led to the selection of a first candidate test for automation. Factors considered in the survey included costs associated with the tests, levels of skill required to conduct the tests, total number of tests conducted, and the need or desirability to increase the number of tests that could be performed.

The fluorescent treponemal antibody-absorption (FTA-ABS) test (2) was selected as the first candidate for automation primarily because it appeared to us that there was a need for a syphilis screening test more sensitive and specific than the present, extensively used Venereal Disease Research Laboratory (VDRL) slide test. It is estimated that there were approximately 38 million standard tests for syphilis (STS) conducted in 1968 in the United States, of which approximately 90 to 95% were the VDRL slide test. Data reported (1) indicate that correlation of test results with diagnostic categories is 77% for the VDRL and 95% for the FTA-ABS. The cost and time required to conduct the FTA-ABS test have prevented its use for large-volume testing; however, it appeared to us that, if automated, it might be found suitable for such screening, thus permitting more accurate case finding and diagnosis and subsequent improvement in control of the disease.

Even with present patterns of use of serological tests, an automated version of the FTA-ABS test would provide a more ready means for detecting patients having false-positive reactivity in cardiolipin tests for syphilis and would enhance the detection of patients with primary, latent, or late syphilis [clinical stages of the disease in which present routine cardiolipin tests are not as sensitive as the FTA-ABS test (2)].

DEVELOPMENT PROGRAM

A development program for the automation of the FTA-ABS test was undertaken in 1967 by Aerojet-General Corp., with technical assistance and liaison from the Verneral Disease Research Laboratory, National Communicable Disease Center, Atlanta, Ga.

During the first phase of the program, 3 January 1967 to 30 November 1967, the manual FTA-ABS test was studied to determine the effects of variations in protocol and the quality of commercially available reagents, and to develop techniques for reagent production. From this study, a protocol for the automated test was established, a process for the production of high quality reagents was proven in pilot lots, and simulated automated tests were conducted successfully. Certain aspects of the reagent development work have been published (3, 4).

Under phase II of the program, 1 December 1967 to 9 August 1968, a "breadboard" instrument was developed and tested. A prototype model was established and four prototype instruments were designed and fabricated. The prototype systems were tested at Aerojet-General Corp., at the Venereal Disease Research Laboratory, National Communicable Disease Center, at San Bernardino County Health Laboratory, San Bernardino, Calif., and at Long Beach Memorial Hospital, Long Beach, Calif., during the period 26 January 1968 to 9 August 1968. A total of 4,500 sera were tested. Results were utilized to make functional improvement during the test program.

Phase III of the program, 10 August 1968 to 21 October 1968, comprised the application of experience from phase II into a production design and the fabrication of one production unit. This unit was then used in a study by the Venereal Disease Research Laboratory, National Communicable Disease Center. In the study, the automated fluorescent treponemal antibody-absorption (AFTA) test was performed on 859 specimens from clinically defined donor groups, and the results were compared with those of the manual FTA-ABS test. The two tests were performed independently by different groups of serologists at the Venereal Disease Research Laboratory. A comparison of the results obtained showed that the agreement between the manual and automated FTA-ABS tests in this study was 87%. Several changes were made in the AFTA test procedure so that it would more nearly approximate the manual procedure. Subsequent test agreement on 201 specimens in clinically defined categories increased to 90%, with the manual FTA-ABS test showing slightly higher sensitivity. Additional refinements are being made which are expected to raise the sensitivity of the AFTA test and to improve the agreement with the manual procedure (G. W. Stout et al., American Public Health Association Annual Meeting, Detroit, Mich., 1968).

Simultaneously with this test program, 15 instruments of the production design were fabricated, and a program was formulated by the National Communicable Disease Center for further field evaluations at California State Department of Public Health Laboratory, Berkeley; Venereal Disease Research Laboratory, National Communicable Disease Center, Atlanta, Ga.; Georgia State Department of Public Health Laboratory, Atlanta; and New Jersey State Department of Public Health Laboratory, Trenton. As of this writing, the Berkeley and National Communicable Disease Center programs are completed and the Georgia and New Jersey programs have been initiated. The results of these test programs will be the subject of individual papers to be published by each testing agency.

INSTRUMENT

The automated instrumentation, known as the SeroMatic System, is comprised of two major items: a processor (Fig. 1) and a microscope attachment (Fig. 2).

The processor is an electro-pneumatically controlled laboratory test instrument which automates the performance of the FTA-ABS test on as many as 200 test specimens per day (7.5 hr of continuous processor operation).

Specimens are processed sequentially, the first specimen being fully processed and ready for microscope examination 85 min, 45 sec after processor startup. Subsequent specimens are fully processed at intervals of 1 min, 45 sec thereafter. Figure 3 shows the process sequence, and Fig. 4 and 5 are internal views of the processor.

The processor is provided with two reservoirs for containing the wash fluids used during specimen processing and with a set of 20 reusable 10-cavity serum-sorbent applicator blocks.

Processor specification. The processor is 35 inches wide × 25.5 inches deep × 22 inches high (89 × 65 × 56 cm) and weighs approximately 120 lb (54.5 kg). Utility requirements are electrical power (120 v, 60 Hz at 10 amp maximum) and air

pressure (40 to 50 psig at 2 ft^3/min). A floor sink or 10-gal (38-liter) reservoir is required for collection of process fluid waste. Acceptable ambient temperature and relative humidity limits are 20 to 30 C and 30 to 90%, respectively.

Reagents required to perform the automated FTA-ABS test are as follows. Antigen: one REDI-FIX antigen slide per specimen. (The slide is comprised of a 25 × 75 × 1 mm thick styrene plastic frame into which is mounted a 22 × 40 mm

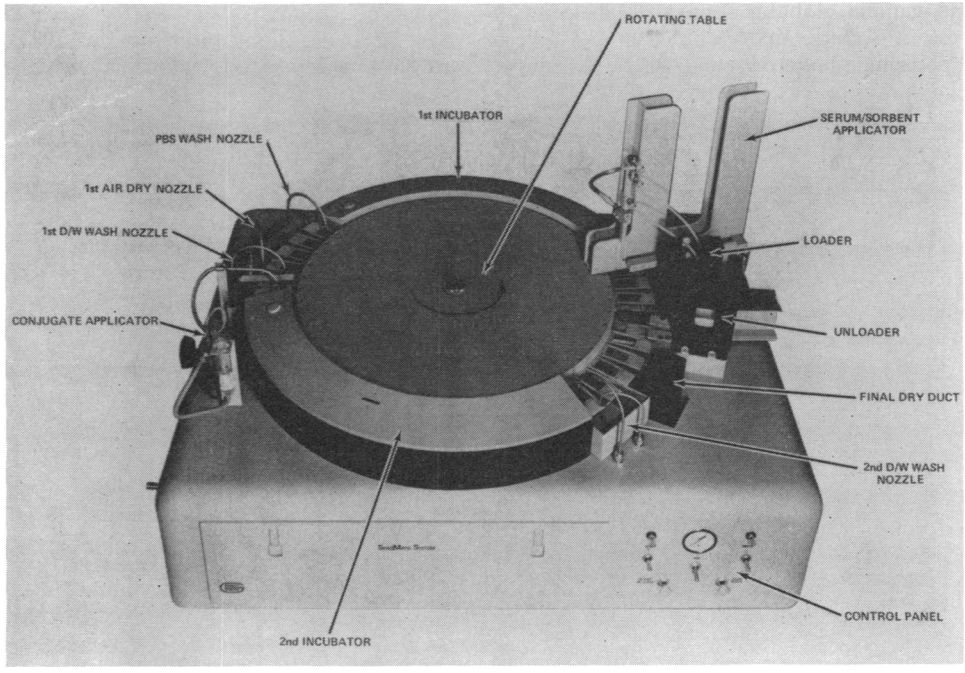

FIG. 1. *Processor.*

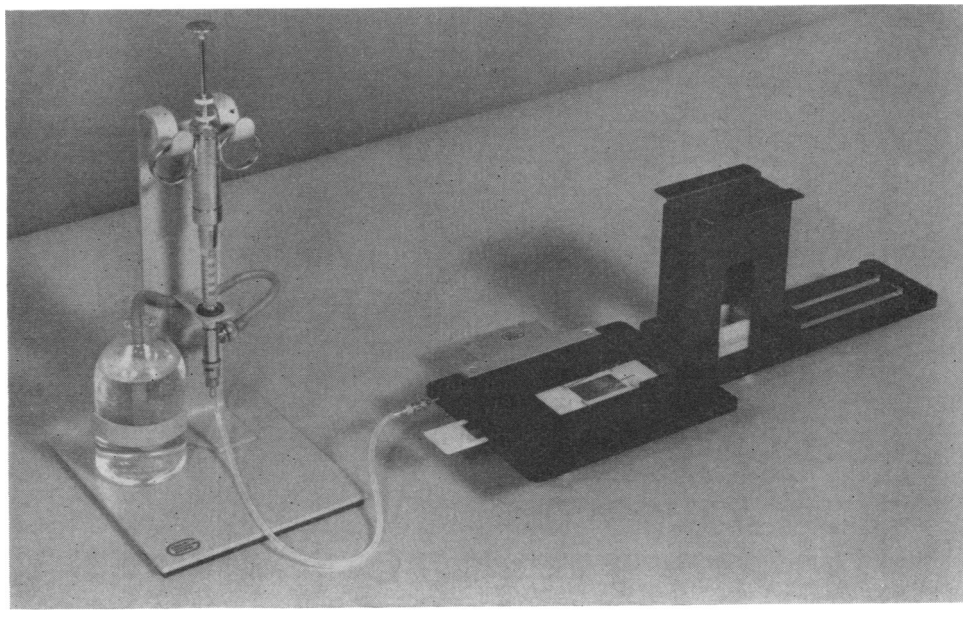

FIG. 2. *Microscope attachment.*

no. 1½ cover glass. On the cover glass is a methanol-fixed 2.5-mm spot of Nichols *T. pallidum* antigen. After fixing, the antigen density is 50 to 100 organisms per high power field.) Sorbent: 0.1 ml per specimen. Patient or reference serum: not less than 0.025 ml per specimen. Titered anti-human gamma globulin conjugate labeled with fluorescein isothiocyanate [diluted in 2% Tween 80 in phosphate-buffered saline (PBS)]: 0.1 ml per specimen. PBS: approximately 20 ml per specimen. Distilled water: approximately 160 ml per specimen.

Process sequence. The proces sequence is graphically illustrated in Fig. 3 and is described below by following one slide through the entire sequence.

At the load station (station no. 1), a REDI-FIX antigen slide is inserted, by the loader mechanism,

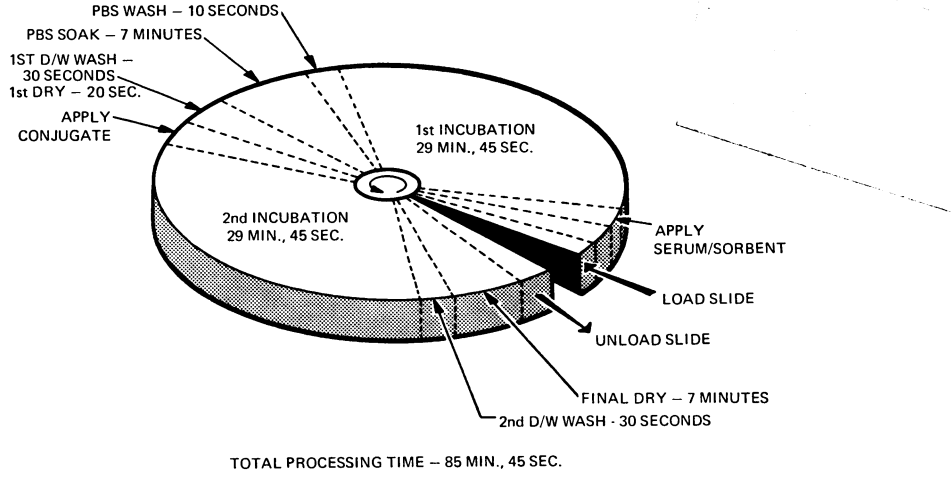

FIG. 3. *Processing sequence.*

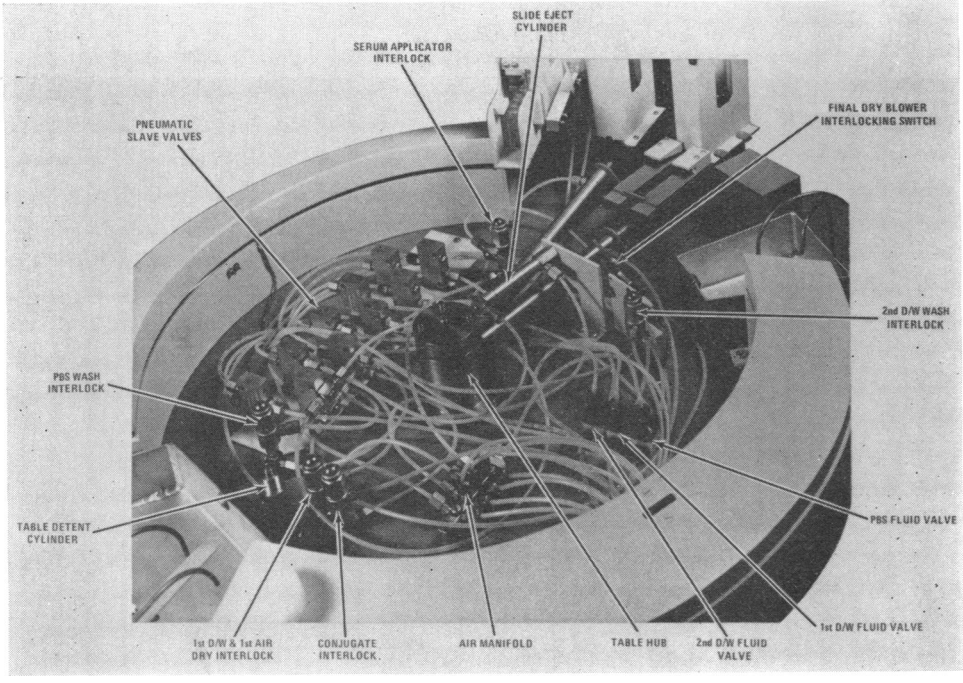

FIG. 4. *Internal top view.*

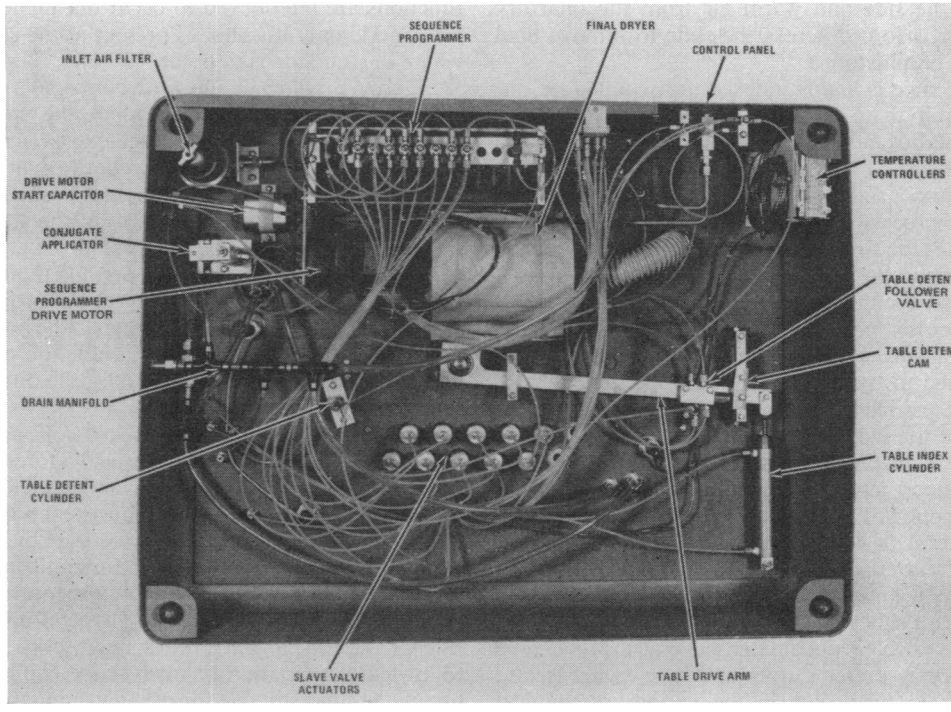

FIG. 5. *Bottom view.*

from a slide-handling magazine into one of 50 slide-holding positions which are equally spaced around the periphery of a circular rotating table.

The rotating table is advanced (indexed) 1/50 revolution (1 station) counter-clockwise every 1 min, 45 sec. Subsequent slides are inserted into adjacent slide-holding positions as these positions are moved into line with station no. 1.

At 3.5 min after a slide has been inserted into a slide holder, it becomes positioned under the serum-sorbent applicator (station no. 3) where the serum-sorbent mixture is forced from a cavity of a serum-sorbent block onto the antigen spot on the slide. This station is provided with a slide-sensing interlock to maintain synchronization of serum specimens with slides.

The slide is then indexed through the first incubator. The first incubator is 17 stations in length; therefore, the slide will remain in the incubator 29 min, 45 sec, during which time the antigen-serum reaction takes place. Incubator temperature is maintained at 37 C.

The first incubation is terminated by indexing the slide out of the incubator and under the PBS wash nozzle. This nozzle applies PBS, *p*H 7.2, onto the slide for a period of 10 sec. Upon termination of the wash, a portion of the PBS wash fluid remains on the slide and in contact with the serum-reacted antigen spot. During the remainder of the time which the slide remains at this station, as well as during the time it spends at the next three stations (approximately 7 min total), PBS soak occurs.

When the table is next indexed (this now being 42 min from the time the slide was inserted into its holder), the slide is brought to station no. 25 where the slide is deflected downward and rinsed with distilled water for a period of 30 sec to remove the PBS and the particulate matter not related to the antigen-serum reaction. Upon termination of the distilled water wash, the air dry nozzle (also located at this station) is activated for 20 sec to remove all distilled water from the top surface of the slide cover glass.

Next, the table is indexed to the conjugate applicator station where a 0.1-ml drop of conjugate is applied to the antigen spot on the slide cover glass.

The slide advances through the second incubator, remaining in the incubator for a period of 29 min, 45 sec. Incubator temperature is maintained at 37 C.

The slide is next indexed to the second distilled water wash station (station no. 44), where a 30-sec distilled water wash is applied to remove residual conjugate.

The slide is then indexed through four stations where large droplets of distilled water are wiped

from the side and warm air from the final dry blower is forced across the slide to remove final traces of moisture.

The slide is finally indexed to the unloader station (station no. 49) where the slide is ejected from the rotating table onto an unload platform. The unload mechanism carries the slide from this platform into a slide unload magazine.

The process sequence is terminated (85 min, 45 sec after the slide was inserted into a slide holder), and the slide is ready for examination with a fluorescence microscope assembly.

Processor control. All time-related operations are controlled by a rotating cam timer which is attached to the underside of the instrument deck and accessible through a hinged panel on the front face of the instrument.

The cam timer is driven by a 1 rev/min synchronous motor and is appropriately geared to rotate one full revolution every 1 min, 45 sec.

A total of 14 cams are attached to the timer shaft. Eleven of these cams are operational, the remaining three are spares. As the shaft rotates, each cam either opens or closes an associated electrical switch or pneumatic valve at a preset time and for a preset duration. These switches and valves, in turn, control the various mechanical functions of the processor.

Certain functions of the process occur during each revolution of the timer cycle, such as table indexing and slide loading. Serum-sorbent application, fluid washes, and conjugate application functions are interlocked to occur during a given timer cycle only if a slide is present at the station where the function is to occur.

Figure 6 graphically illustrates the operation of all timer-controlled functions where the "home" position of the cam timer is specified as reference. Those functions shown as solid lines occur each time cycle. Those functions shown as dashed lines occur only when a slide is present at the stations where such functions are to occur.

The timer is of a design that permits rapid and simple alteration of the timer cycle program, where desired. This includes changing the point in time, during a timer cycle, that a function occurs and, in the case of the three washes, the duration of the washes.

Microscope attachment. The microscope attachment, when installed onto a dark-field fluorescence microscope, permits REDI-FIX antigen slides to be visually examined at a rate consistent with the maximal daily capacity of the processor by minimizing the handling of slides and minimizing the necessary manipulation of microscope controls by the operation. Figure 7 shows a typical installation of the attachment onto a microscope. [The following combination of microscope equipment has been found suitable for use in examination of processed slides: illumination system consisting of an Osram HBO-200 ultraviolet light source and 6-v 30-w tungsten light source; excitation filtration consisting of a 2-mm thick KG 1 (heat absorption) filter, a 2-mm thick BG-12 (or equivalent)

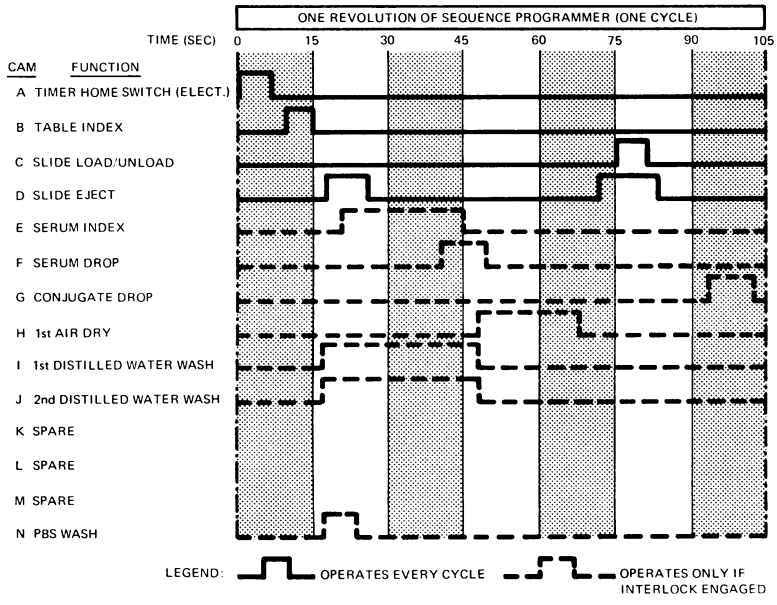

FIG. 6. *Timing control diagram.*

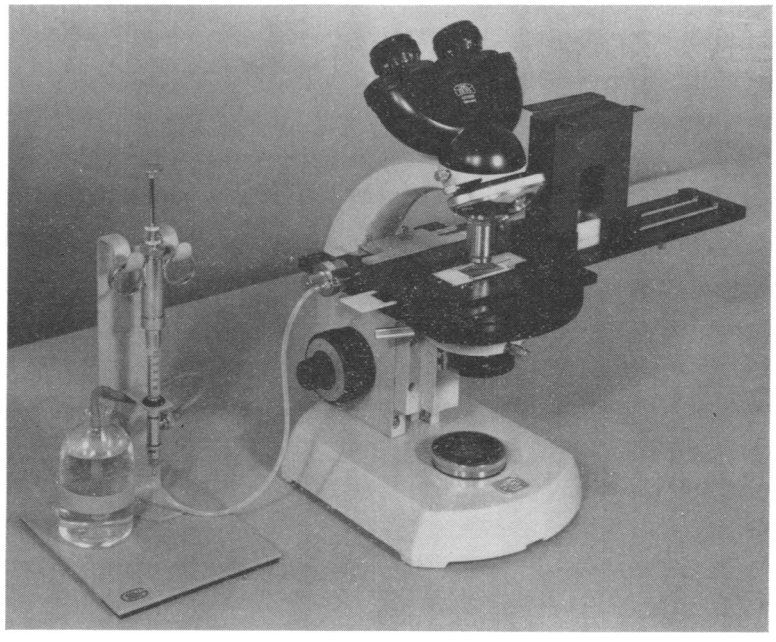

FIG. 7. *Microscope with attachment and glycerol feed assembly.*

filter and a 2-mm thick BG-38 (or equivalent) filter; a 2-mm thick OG-1 (or equivalent) barrier filter, an immersion-type dark-field condenser having a numerical aperture of 1.20; a 40 × high dry objective with a numerical aperture of 0.65; and a 10 × eyepiece.]

The attachment accepts a processor unloader magazine containing the processed slides. The slides are accurately positioned under the objective lens of the microscope by the operator. The design of the attachment is such that the antigen spot on the slide is in sufficient focus to require only very slight adjustment of the microscope fine focus control to obtain a sharp field image.

A glycerol feed assembly is provided with the attachment (*see* Fig. 7). When a slide has been positioned under the objective, the operator depresses and releases the plunger of this assembly to glycerol-couple the slide cover glass to the dark-field condenser.

After examining a slide, the operator positions the next slide under the objective, thereby pushing the previous slide clear.

The unique feature of this method is that the REDI-FIX slides are placed in the attachment in an inverted position; that is, the reacted antigen spot is on the lower side of the slide. By handling slides in this manner, the need for installing a cover glass over the specimen (as with conventional slides in fluorescence microscopy) is eliminated.

DISCUSSION

The feasibility of utilizing the automated instrumentation in the performance of routine immunofluorescence tests has been demonstrated. This has been accomplished by successfully automating the repetitious, time-consuming, and often tedious steps, including those necessary during slide readout, associated with manually performed tests.

Test results to date give general indication that the FTA-ABS test has been successfully automated. The subject instrument will permit an increase in the total number of tests which can be performed per unit operator time to a point where the test may be used more extensively for screening for syphilis.

Preliminary experiments (Norins et al., *to be published*) have demonstrated the feasibility of using this instrumentation in the detection of antibodies to malaria parasites, toxoplasma organisms, and nucleoprotein (antinuclear factor). Experimentation continues in an effort to discover additional immunofluorescence tests which can be similarly automated.

It is felt that the inherent functional flexibility of this instrument may be a further measure of its value, providing investigators with a fertile tool in the field of developmental research.

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