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AUTOMATED AGGLUTINATION REACTIONS FOR DETECTION OF AUSTRALIA ANTIGEN, *T. CRUZI* ANTIPOLYSACCHARIDE ANTIBODIES, AND ANTI-*SALMONELLA* FLAGELLAR ANTIBODIES[1]

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Procedures have been developed that permit automated screening of serum samples for antibodies linked to Chagas' disease, hepatitis, and Salmonella infections. In cases where large numbers of samples are being processed, such automated procedures may save significant time and effort.

Introduction

The work reported here was performed in order to develop a standardized automated technique for making several serologic evaluations important in our part of the world. It is hoped that this technique will permit these evaluations to be made at increased speed and reduced cost.

A number of techniques have been described and used for detection and analysis of Australia antigen, anti-*Trypanosoma cruzi* antibodies, and anti-*Salmonella* flagellar antibodies. For the most part these techniques are relatively simple and produce sufficiently specific and sensitive results. Nevertheless, they lack the speed desired for undertakings that process large numbers of samples—such as epidemiologic investigations or blood screening work by blood bank serology departments seeking to prevent transmission of disease agents.

To help develop a faster technique, we decided to adapt the automated Technicon Autoanalyser equipment designed to carry out serological reactions for the detection of syphilis (*8*). Several other groups of researchers had previously studied and modified this automated system for purposes of carrying out additional standardized tests (*4, 6, 9*). Our work consisted, first, of making some operational modifications in the system and then adapting two tests (passive hemagglutination and bacterial agglutination) to the modified system. After that, tests for Australia antibody, anti-*T. cruzi* antibody, and anti-*Salmonella* flagellar antibody were carried out to see if the modified system could reliably perform these tests more easily and rapidly than they could be performed with manual techniques.

For detection of *T. cruzi* antipolysaccharide antibodies, automated passive hemagglutination, manual passive hemaglutination, and manual complement fixation tests were performed with serum samples obtained from 60 subjects with Chagas' disease and 14 control subjects. Automated passive hemagglutination was also used to detect Australia antigen in 50 serum samples from blood donors, the results then being subjected to qualitative and quantitative comparison with the results of two other tests, manual passive hemagglutination and counterimmunoelectrophoresis. In addition, anti-*Salmonella* flagellar antibodies were analyzed by means of automated bacterial agglutination, and the results of this procedure were compared with those obtained with the manual technique normally used for this purpose.

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Materials and Methods

Equipment

A diagram of the modified Technicon system used to perform all the automated tests is shown in Figure 1.

Bacterial Agglutination Tests

The bacterial agglutination tests, both manual and automated, employed 197 serum samples from normal blood donors. All the serum samples were collected under aseptic conditions and were kept at a temperature below -20°C until used. Thirty-nine of these serum samples were tested against flagellar antigen b of *Salmonella paratyphi* B; 43 were tested against flagellar antigen i of *Salmonella aberdeen*; 35 were tested against flagellar antigen 1.2 of *Salmonella morotai*; 39 were tested against flagellar antigen mt of *Salmonella oranienburg*; and 41 were tested against flagellar antigen d of *Salmonella typhi*.

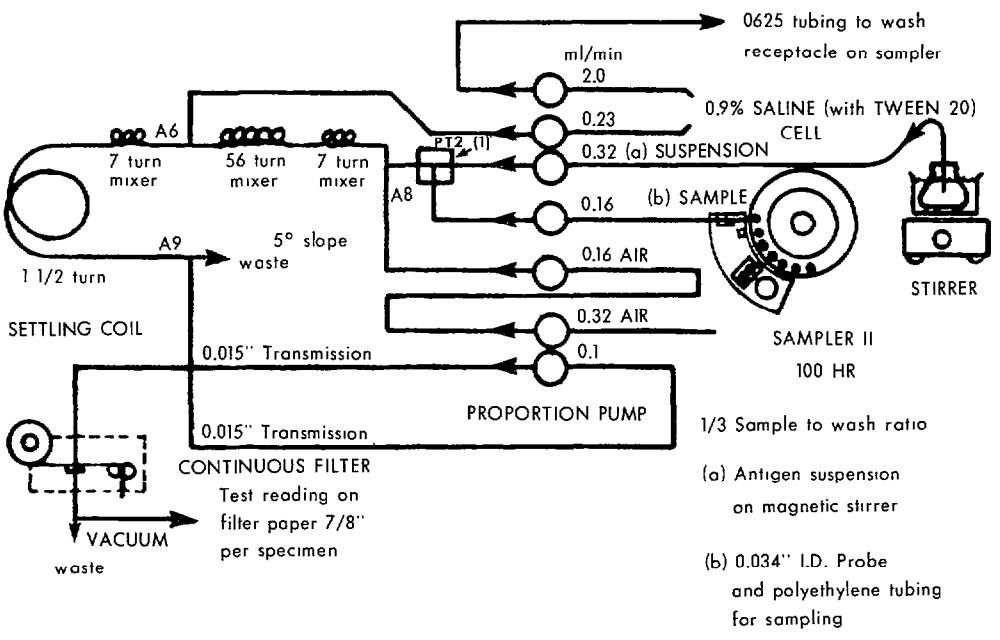
Salmonellas suspended in formaldehyde were employed as antigen. Unstained antigens were used in the manual tests, while for the automated tests the antigens were stained as follows: Crystal violet (0.2 ml) was added to 10 ml of antigen, and the mixture was centrifuged four times, each time at 5,000 r.p.m. for 15 minutes. The final product was resuspended in 5 ml of a 0.9 per cent salt solution containing 1 per cent basic fuchsin.

While the automated tests were performed with the forementioned equipment (see Figure 1), the manual tests were performed by combining sera and suspended antigens on plates and observing whether agglutination occurred.

Passive Hemagglutination Tests

All of our passive hemagglutination tests,

Figure 1. Flow diagram of operations performed by the modified equipment used in the automated tests.



used to detect both *T. cruzi* antibody and Australia antigen, employed human erythrocytes treated with formaldehyde and tannic acid. Those erythrocytes used to test for *T. cruzi* antibody (obtained from type O, Rh negative blood) were sensitized with polysaccharide *Trypanosoma* antigen, while those employed in the tests for Australia antigen came in the form of a sensitized and freeze-dried product (Hepanosticon ®) obtained from the commercial laboratory Organon Ltda. (*5*). This latter product was used at twice the recommended concentration in the automated tests and at the recommended concentration in the manual tests.

Both sets of automated tests were performed with the forementioned equipment (see Figure 1). The manual hemagglutination tests for *T. cruzi* antibody employed microtiter plates according to the technique described by Takeda, et al. (*7*). The tests for Australia antigen employed the technique standardized by Organon, Ltda.; aside from the concentration of erythrocytes, reagents used in the manual and automated tests for Australia antigen were the same.

Complement Fixation Test for T. cruzi *Antibody*

This test was performed according to the technique described by Almeida (*1*). This method calls for evaluating reactions in terms of the serum (or antigen) needed to produce 50 per cent lysis, as indicated by a pair of isohemolytic curves found by using arithmetic coordinates. The technique is thoroughly described in the original work. Reagents employed in this test, including methylic antigen, complement, erythrocytes, and hemolysin, were obtained from Lio-serum Ltda., the usual suppliers of these products.

Counterimmunoelectrophoresis to Detect Australia Antigen

This test was done on agar-gel plates with the standard technique, reagents, and equipment specified by the Hyland Division of Travenol Laboratories (*3*).

Results

The results of the manual and automated hemagglutination tests for *T. cruzi* antibody were identical. A comparison of these results with those obtained by manual complement fixation of the same serum samples appears in Table 1.

Qualitative results of the three kinds of tests performed to detect Australia antigen are shown on Table 2. We also made a quantitative comparison of titers obtained with nine sera, the results of which are shown in Table 3. To guard against false positive reactions, all the serum samples tested were subjected to further treatment with absorbent Hepanosticon ® and were then retested against erythrocytes sensitized with antibodies against Australia antigen. This procedure did not turn up any false positive responses or any titers lower than those previously recorded.

The results of the two sets of bacterial agglutination tests performed to detect anti-*Salmonella* flagellar antibodies are shown in Table 4.

Discussion

Hemagglutination Tests

Various techniques have been described that are designed to facilitate detection of Australia antigen and antibodies against *T. cruzi*. We have found, however, that difficulties arise when such entities as blood bank serology departments need to process a large number of samples. It is our opinion that such needs can best be met with automated passive hemagglutination.

Analyzing the results obtained to date, we have concluded that the automated passive hemagglutination test for the detection of

Table 1. Comparison of qualitative results obtained with automated and manual passive hemagglutination and complement fixation tests for *T. cruzi* antipolysaccharide antibodies.

	Total No. of sera	Automated and manual hemagglutination		Complement fixation	
		+	−	+	−
Sera from Chagas' disease patients	60	56	4	58	2
Sera from control subjects	14	0	14	0	14

Table 2. Comparison of qualitative results obtained with automated and manual passive hemagglutination and counterimmunoelectrophoresis tests for Australia antigen.

	No. of sera	Passive hemagglutination tests			
		Automated		Manual	
		+	−	+	−
Positive by CIE	40	39	1	39	1
Negative by CIE	10	2	8	1	9

Table 3. Comparison of titers obtained by testing nine serum samples for Australia antigen with automated and manual passive hemagglutination and counterimmunoelectrophoresis.

Serum sample No.	Titers obtained from:		
	Counterimmuno-electrophoresis	Automated passive hemagglutination	Manual passive hemagglutination
1	4	16	16
2	4	16	8
3	8	8	8
4	16	64	16
5	16	64	16
6	16	32	32
7	32	32	16
8	32	32	32
9	64	64	32

anti-*T. cruzi* antibody and Australia antigen is specific, quite precise, and very quick. Regarding the latter point, this equipment is capable of making 100 determinations per hour, while the manual methods require three hours or more to perform that task. This difference is important if numerous samples are involved.

Care must be taken in preparing erythrocytes for the *T. cruzi* antibody test, but the procedure involved is not difficult. Regarding the test for Australia antigen, the avail-

Table 4. Comparison of qualitative results obtained with automated and manual agglutination tests for anti-*Salmonella* flagellar antibodies.

Salmonella flagellar antigen	No. of sera tested	Test results			
		Manual agglutination		Automated agglutination	
		+	–	+	–
b	39	28	11	26	13
i	43	33	10	32	11
1.2	35	30	5	30	5
mt	39	18	21	16	23
d	41	21	20	19	22
Total	197	130	67	123	74

ability of commercially prepared erythrocytes helps to assure both the desired exactness and subsequent reproducibility of the results.

Results of both these automated tests can be read more easily and precisely than those of the manual tests, because a positive reaction produces a dark brown color on the filter paper strip that is easily distinguished from a negative reaction.

Overall, it is the authors' belief that these automated hemagglutination techniques are well-suited to the needs of blood bank serology services seeking to prevent transmission of blood donor diseases such as Chagas' disease and serum hepatitis. It is also felt that other hemagglutination techniques can be effectively adapted to the automated system employed.

Bacterial Agglutination

Similarly favorable results were obtained with the automated tests for anti-*Salmonella* flagellar antibodies. That is, the technique was found to be precise, specific, and quick, permitting up to 100 determinations per hour. Approximately twice that time is normally required to make 100 determinations with manual methods. As in the previous cases, this saving of time would seem to offer an important advantage for undertakings such as epidemiologic investigations where great numbers of determinations are made. The previously described preparation of stained antigen for this automated test is a very simple procedure that involves no significant loss of efficiency or time.

Again, the test results can be read easily and more precisely than the manual test results; positive results color the filter paper blue, while negative results color the paper red.

Concluding Remarks

With regard to laboratory costs of the automated equipment, our experience in processing large numbers of samples has shown that savings in time and man-hours do in fact justify the costs involved. We also think it likely that similar automated techniques can be devised to detect antibodies against other disease agents. It is our intention to follow up on the work reported here with quantitative study of the tests described and also with investigation of other antigen-antibody systems, the goal being to promote research and evaluation work now hindered by the difficulty of processing large numbers of samples.

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SUMMARY

This article describes the development and testing of automated systems for detecting Australia antigen, antibodies against *Trypanosoma cruzi*, and anti-*Salmonella* flagellar antibodies. The basic techniques used were passive hemagglutination in the first two instances and bacterial agglutination in the third. All three procedures employed a modified version of automated equipment currently used to detect syphilis.

Trials indicated that the new automated methods provided specific, easily readable, and rapid results. These trials were conducted with sera from 50 blood donors for detection of Australia antigen, sera from 60 Chagas' disease patients and 14 control subjects for detection of antibody against *T. cruzi*, and sera from 197 blood donors for detection of anti-*Salmonella* flagellar antibodies. It was concluded that the new techniques offer definite advantages if large numbers of samples are being processed, and that they seem well-suited to use in blood bank screening activities and epidemiologic investigations.

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