QUICK SLIDE TEST FOR ESTIMATING THE PATHOGENICITY OF STAPHYLOCOCCI

The Comparison of Coagulase Activity with Clumping in Citrated Normal Human Plasma*

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Coagulase positive staphylococci are almost constantly clumped if an attempt is made to suspend them in normal citrated human plasma in tubes or on slides. To investigate the dependability of this relationship, we conducted a number of tests with 547 staphylococci of various origin, such as blood, body fluids, skin lesions, throat and nose secretions, and the air of hospital wards.

This paper gives the details of our tests and results which, in fact, are similar to the results obtained by other investigators elsewhere in the world.

Introduction

Numerous tests have been recommended for estimating the pathogenicity of a given strain of staphylococci.[1,2] Since some of them are laborious, expensive and complicated and others time consuming, the tests commonly employed in the average laboratory are mannitol fermentation, hemolysin and coagulase production. It is believed that the last mentioned test gives the most reliable information about pathogenicity in a relatively short time.

Cruickshank[3] reported that all pathogenic strains of staphylococci, no matter whether they are aureus or albus types, constantly produce staphylocoagulase. Christie and Keogh[4] also found that all staphyloccci which are related to a pathological process possess this ability, and that it is not possible to make a true connection between a coagulase negative staphylococcus and a pathological process.

During a previous survey made by one of us (M. A.)[5] it was noted that some staphylococci were rapidly clumped in the coagulase test

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tubes during inoculation and a high proportion of them were seen to be coagulase producers. Coagulase negative strains did not show this phenomenon and both coagulase positive and negative strains gave quite homogeneous suspensions in saline. This relationship stimulated us to search the literature on the matter and to make comparative studies with the staphylococci isolated later on.

Much [6] was the first author who noted the reaction of "clumping" if an attempt is made to suspend coagulase positive staphylococci in citrated human plasma. Birch-Hirschfeld, [7] making comparative studies with over a hundred strains, found that thick suspensions of coagulase positive staphylococci were rapidly clumped if mixed with plasma. Cadness-Graves et al. [8] confirmed the parallelism between clumping in a drop of citrated plasma and the classical tube coagulase test results. It has even been noted that some weak positives, which might be missed in tube tests, could more easily be detected with the slide technic. [9]

It has been recommended that the slide method should not be adopted without making a series of tests in parallel with the tube test. So, before going ahead with the slide test in routine examinations, it was necessary also for us to conduct some comprehensive tests with the strains and the conditions existing in this country.

Materials and Methods

Plasma Used: Plasma was obtained from whole blood from the Red Crescent's Blood Collecting Center. These blood samples were collected into classical ACD solutions in the usual manner. Saline dilutions of 1:1 to 1:256 were used for the slide test, and 1:4 for the tube tests.

Test Tubes: The tubes used were 8×75 mm. in size and in each was placed 1 cc. of plasma dilution. Test results were read after 4 and 24 hours of incubation at 37 C. Any degree of coagulation was taken as evidence of coagulase production.

Recording of Clumping: Only prompt and strong clumping within 20 seconds was accepted as real clumping. No attention was paid to late weak reactions. In the case of a positive reaction, clumping is so clear that it can not be missed by a laboratory worker with a little experience.

Inoculation: Instead of making broth cultures or saline suspensions, material taken by a loop from a plate or from a subculture was used directly for inoculation. Using a flamed loop in each case, material was put first on a dry place on the slide (or on the inside wall of the tube when the tube test was performed) near the plasma, crushed thoroughly and then mixed with the plasma so that the existence of big masses of the material could easily be avoided.

Controls: In case of clumping, another test was performed using saline in the same manner, on the slide or in tubes. Positives were recorded as "auto-agglutination." If this second test was negative, the saline drop was allowed

to dry, stained with Gram's stain and examined microscopically for staphylococci. In addition, one uninoculated plasma tube was incubated with each series of tests.

Results

1. For the first test, single colonies of staphylococci on plates were chosen. From the same colony, a stained slide and a subculture were made on agar slants. On the following day, a tube coagulase test was performed and a record made of the results of these tests and whether there was clumping during inoculation. Positive strains gave typical "snow screen" appearances as is seen in figure 1. Negatives and controls remained homogeneously turbid. Results are shown in table 1.

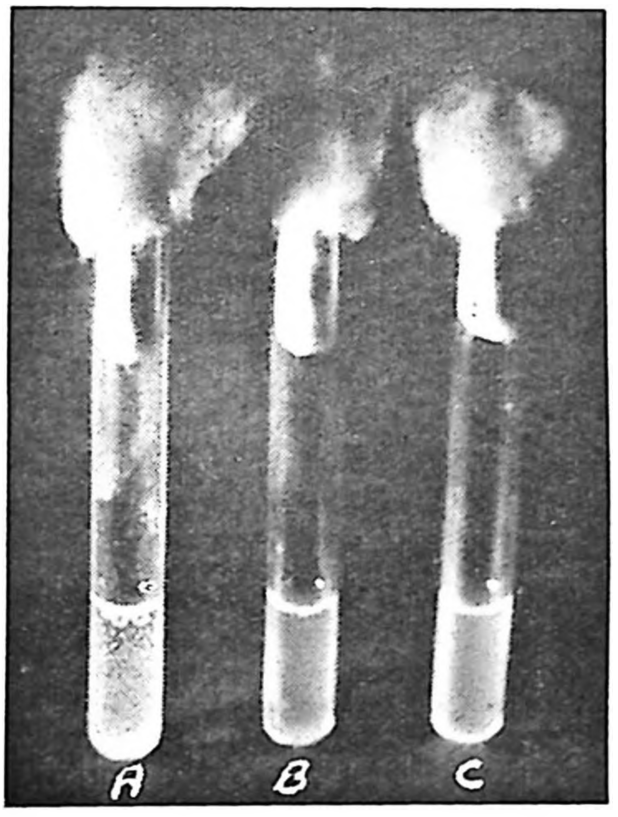

Fig. 1. — Semi-schematic picture showing immediate appearance in tubes when staphylococci are mixed with the citrated plasma in a 1:4 dilution. A: Coagulase positive strain. B: Coagulase negative strain. C: Saline control. (Note the "snow screen" appearence in tube A and the homogeneous turbidity in tubes B and C.)

It is seen that 98.6 per cent of positives and 95.9 per cent of negatives gave consistent results in both tests. Both technics gave consistent results for a total of 525 strains. (97.4 per cent).

2. Of these 547 strains, 337 were tested on slides with a drop of plasma, 1:4 dilution, and coagulase tests were made in parallel. (Fig. 2). Results are given in table 2.

TABLE 1.

THE CORRELATION BETWEEN CLUMPING IN TUBES AND COAGULASE PRODUCTION

TOTAL NUMBER TESTED	547	COAGULASE WITHIN 24 HOURS		AUTO-AGGL. (+)	STRAINS GIVING INCONSISTENT REACTIONS	
		POSITIVE	NEGATIVE		NUMBER	%
CLUMPING (+)	302	290	12	8	4	1.3
CLUMPING (—)	245	10	235	—	10	4

From that table it is clear that the two tests gave consistent results in 100 per cent of the cases both for coagulase positive and negative strains. According to these results it should easily be possible to determine the coagulase production of 97.3 per cent of the strains tested within 20 seconds, without waiting for the results of tube coagulase tests. It is evident that for these 9 auto-agglutinable strains it is necessary to make a tube coagulase test before reaching a final conclusion.

TABLE 2.

THE CORRELATION BETWEEN THE SLIDE TESTS AND TUBE COAGULASE TESTS WHEN PURE CULTURES OF STAPHYLOCOCCI WERE USED

TOTAL NUMBER TESTED	337	COAGULASE WITHIN 24 HOURS		AUTO-AGGL. (+)
		POSITIVE	NEGATIVE	
CLUMPING (+)	210	201	9	9
CLUMPING(—)	127	—	127	—

3. In the first two series of tests, pure cultures were used. But for the third series mixed cultures were used and bacteria which were taken directly from the plates were used both for slide and tube coagulase tests. In taking the material, care was taken to select mostly staphylococcal colonies. Results are summarized in table 3.

It will be noted that with mixed cultures also, both tests gave rather parallel results for 202 out of 207 (98 per cent). It was necessary to wait for the results of tube tests in only 1.4 per cent of the cases. If the results in tables 2 and 3 are studied together, it will be seen that for 530 strains out of 547 (96. 8 per cent) it was possible to reach a decision within 20 seconds. This is, of course, a result which could not be overlooked.

4. During our experiments, 340 strains were tested with 1:1 to 1:256 dilutions of plasma on slides, the end point of clumping was

recorded, and parallel tube tests were made. It was seen that there was no correlation, whatsoever, between the titer which causes clumping and the time, size or consistency of the coagulum formed.

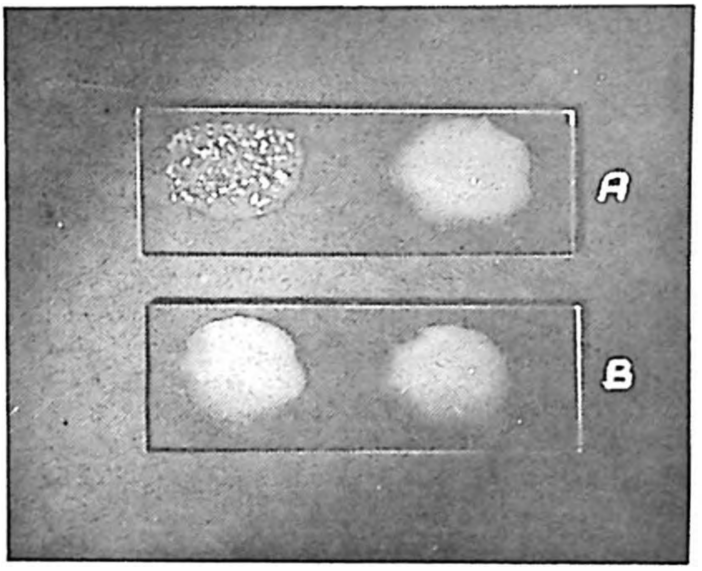

Fig.2. — Immediate appearance on slides when the staphylococci are mixed with plasma and saline drops. A:Coagulase positive staphylococcus, B: Coagulase negative staphylococcus. (The drops on the left are 1:4 diluted plasma and the right hand drops are saline. Note clear clumping in the plasma drop and homogeneous turbidity in the saline drops.)

TABLE 3.

THE CORRELATION BETWEEN SLIDE AND TUBE TESTS WHEN MIXED CULTURES WERE USED

TOTAL NUMBER TESTED	210	COAGULASE WITHIN 24 HOURS		AUTO-AGGL. (+)	STRAINS GIVING INCONSISTENT RESULTS	
		POSITIVE	NEGATIVE		NUMBER	%
CLUMPING (+)	103	96	7	3	4	4
CLUMPING (—)	107	1	106	—	1 *	0.9

* Although repeated twice, showed no clumping in tube or on slide. This is a real discrepancy.

5. Pigment type was recorded after 48 hours of incubation at 37 C. on Bacto-Chapman Stone Medium.[10] The results are as follows in table 4.

These results are in agreement with the common idea that most of the coagulase producers are among the aureus strains and only a small proportion of albus strains produce coagulase. But it should be noted,

that there is no complete correlation between pigment formation and pathogenicity so far as the coagulase test results are concerned. Some aureus strains should be considered saprophytic, while some albus strains are real pathogens.

TABLE 4.
THE CORRELATION BETWEEN PIGMENT TYPE AND COAGULASE PRODUCTION

AUREUS		ALBUS		CITREUS	
249		290		8	
COAGULASE (+)	%	COAGULASE (+)	%	COAGULASE (+)	%
233	93.5	62	21	4	*

* Computation would be of no value since the number of strains is too small.

6. Ninety-six and nine-tenths per cent of the coagulase positive strains gave a positive result within 4 hours and the remainder within 24 hours. If the test is limited to 4 hours' incubation a false result in about 3 per cent of the cases is to be expected.

7. During the experiments, 9 different plasmas were used. All gave positive clumping with positive control strain and no clumping with negative control strain. It is our plan to continue the tests in the future with over a hundred plasma samples taken from people at different ages, and from animals.

Discussion

Cadness-Graves et al. [8] found in their experiments that there was parallelism between the tests in 99.5 per cent of cases. In another series, if they counted the late positives (those that appeared after 20 seconds) among the negatives the rate of correlation would be 87 per cent for 558 coagulase positive strains. They recommended that all slide negative strains should be tested in coagulase tubes.

Our tests showed that if pure cultures of staphylococci are used, the rate of correlation is 100 per cent. So,if pure cultures are used and all auto-agglutinable strains are tested in coagulase tubes, in our opinion, it is not necessary to test all negative strains again in tubes. Since the rate of correlation between slide clumping tests and tube coagulase tests for mixed cultures proved to be about 98 per cent, the slide test can also

be used directly for mixed cultures from plates, in estimating the pathogenicity of staphylococci. In this case it would be advisable to dip the loop mostly into typical staphylococcal colonies or those that seem to resemble them.

It is well known that all laboratory tests, even the most dependable ones, give false results to some extent. Clumping in citrated human plasma, if it is used with certain precautions, can be a very valuable test in estimating the pathogenicity of staphylococci. It is a simple and inexpensive procedure whose use would effect savings in both time and material.

Especially in those cases where a pure culture is usually grown, such as cultures from blood, urine, body fluids and skin lesions, a quick decision would be of priceless value both for laboratory workers and for clinicians. Since coagulase production is believed generally to be the most important criterion for pathogenicity, and may well be the first important one, and since there is a direct and very close correlation between coagulase production and clumping in citrated plasma, it is quite possible to determine the pathogenicity of a given strain of staphylococcus within 20 seconds, almost with certainty. According to our tests, in only 1.4 per cent of the cases, was it necessary to make the tube test in order to reach a conclusion. Slide tests were effective in all others.

The slide test: (a) saves time (20 seconds or so, instead of 4 to 24 hours),
(b) saves plasma (1 drop of diluted plasma is enough for about 15 - 20 tests),
(c) saves material in general.

Summary

Coagulase positive staphylococci are rapidly clumped in normal citrated human plasma (or in its dilutions) when an attempt is made to suspend them in tubes or on slides. Coagulase negative strains give quite homogeneous suspensions. The difference between positive and negative strains is very clear.

Experiments made by other authors, as well as our tests, have shown that the parallelism between coagulase production and slide clumping tests is evident and the rate of correlation is rather high. It was always above 97 per cent and was 100 per cent when pure cultures of staphylococci were used.

Since coagulase production is believed to be the most important criterion for pathogenicity, the slide test can be used in estimating the pathogenicity of staphylococci and gives satisfactory results within 20 seconds. The slide test is a cheap, time saving, dependable test.

In the text, the technic of our tests and results and also the correlation between the type of pigment and of coagulase production is given.

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