

THE USE OF SEMI-SOLID AGAR FOR THE DETECTION OF BACTERIAL MOTILITY¹

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Although the hanging-drop method for the detection of bacterial motility can be employed successfully, the procedure has several distinct disadvantages. It is so tedious that the determination of this bacterial characteristic is frequently neglected in the routine laboratory. Furthermore, the results are often uncertain because it is difficult to observe motility when only a few of the cells in a culture are motile. Finally, it is necessary to provide relatively young cultures for the examination.

The use of semi-solid media for the determination of bacterial motility, on the other hand, eliminates the shortcomings of a hanging-drop technique. The results are macroscopic. They are cumulative, thereby particularly qualifying the method for use in the routine laboratory, where examinations cannot always be carried out at a specific time. Moreover, this method practically eliminates the possibility of overlooking motility when only a small proportion of motile cells are present, because the localized out-growths, which occur wherever motile cells are deposited along the stab, can hardly escape notice.

Semi-solid media have been employed for many years in the study of bacterial motility. Rosenthal (1895) reported marked differences in the size and shape of bacterial colonies which had developed in semi-solid nutrient gelatin. Klie (1896) called attention to the spreading and thread-like appearance of colonies

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of typhoid and colon bacilli in a similar medium. Hiss (1897 and 1902) introduced semi-solid media which were designed to distinguish between typhoid and colon bacilli in either poured plates or stab cultures. Levine (1916) employed semi-solid agar (nutrient broth with 0.5 per cent agar) in culture tubes to determine motility in the *Escherichia-Aerobacter* group. Li (1929) used a semi-solid medium (meat-infusion bouillon plus 0.5 per cent agar and 8 per cent gelatin) in poured plates for the differentiation of motile and non-motile types of the "hog cholera bacillus." Recently, Jordan, Caldwell and Reiter (1934) employed this medium in poured plates to study the motility of *Salmonella Schottmülleri*. Other workers, too, have made use of semi-solid media for the detection of bacterial motility, as evidenced by scattered statements in the literature that hanging-drop results were checked by means of stab cultures in semi-solid media.

During the course of current studies on *Escherichia-Aerobacter* "intermediates," we (1935) obtained perfect correlation between the results of motility determinations made in hanging-drop preparations and in semi-solid agar. Since we knew of no comprehensive investigation comparing the results of the two methods, we undertook such a study. This paper presents the results.

METHODS AND MATERIALS

A total of 1,051 cultures, representing more than 60 species was employed. This number included 665 strains, chiefly members of the *Escherichia-Aerobacter* group, which had been isolated from human urine and feces within 6 months prior to the investigation. The other cultures were selected from the laboratory collection.

The semi-solid medium used in the study was composed of 0.3 per cent meat extract, 0.5 per cent peptone and 0.5 per cent agar. It was adjusted to pH 6.8 to 7.2. It was dispensed in culture tubes and sterilized in the autoclave. Inoculations were made by the stab method with a straight needle. The source cultures were on agar slants or in broth. Incubation was carried

out at 37°C. for 6 days, unless positive results were produced sooner. Hanging-drop preparations were made from either nutrient broth or 2 per cent peptone water cultures, incubated at 37°C. for from 8 to 10 hours; 12 to 15 hours; and 18 to 24 hours.

RESULTS

In the semi-solid agar, motility was manifested macroscopically by a diffuse zone of growth spreading from the line of inoculation. In certain instances, especially with *Aerobacter cloacae* and the motile strains of the *Proteus* group, the growth diffused throughout the medium.

The results of the tests for motility in semi-solid agar and in hanging-drop preparations are recorded in tables 1 and 2. In

TABLE 1
Motility of 1,051 cultures in semi-solid agar and in hanging-drop preparations

MOTILITY	SEMI-SOLID AGAR						HANGING DROPS
	1 day	2 days	3 days	4 days	5 days	6 days	
+	516	538	538	538	538	538	517
-	533	505	496	488	485	481	534
(Nodular)	2	8	17	25	28	32	

hanging-drop preparations, 517 cultures were motile (table 1). In semi-solid agar, after one day, 516 were motile and, after two days, 538 (including all cultures motile in hanging-drop preparations) were motile. Thus, after one day the agar yielded results essentially identical with those of the hanging-drop method; but, after two days, it elicited 4 per cent more positive tests. The only differences between the results of the two methods were found with a single spore-forming culture and certain strains of the *Escherichia-Aerobacter* group, most of which were recently isolated cultures (table 2). A few agar cultures of the *Escherichia* group, after two days, showed evidence of motility by the formation of nodular outgrowths along the stab. Further incubation failed to alter the type of growth in these instances. Isolations from these nodules, and from the non-diffused stab yielded, respectively, motile and non-motile strains

TABLE 2

Motility of various cultures in semi-solid agar and in hanging-drop preparations

ORGANISMS	MOTILITY	SEMI-SOLID AGAR			HANGING DROPS
		1 day	2 days	3-6 days	
<i>Aerobacter</i>	+	24	26	26	25
	-	88	86	86	87
<i>Escherichia "communis"</i>	+	208	213	215	213
	-	188	169	163	185
	(Nodular)	2	6	20	
<i>Escherichia "communior"</i>	+	95	97	98	98
	-	77	73	62	74
	(Nodular)	0	2	12	
<i>Escherichia-Aerobacter "intermediates"</i>	+	78	80	80	80
	-	16	14	14	14
<i>Salmonella</i>	+	46	46	46	46
	-	56	56	56	56
<i>Eberthella</i>	+	11	11	11	11
	-	2	2	2	2
<i>Shigella</i>	+	0	0	0	0
	-	45	45	45	45
<i>Alcaligenes</i>	+	4	4	4	4
	-	0	0	0	0
<i>Proteus</i>	+	35	35	35	35
	-	8	8	8	8
<i>Serratia</i>	+	7	7	7	7
	-	0	0	0	0
<i>Chromobacterium</i>	+	1	1	1	1
	-	0	0	0	0
<i>Pseudomonas</i>	+	6	6	6	6
	-	0	0	0	0
Cocci.....	+	0	0	0	0
	-	40	40	40	40
<i>Bacillus</i>	+	11	11	11	10
	-	3	3	3	4

identical in other cultural characteristics. Thus, motile strains have been obtained from some cultures which were thought to be non-motile on the basis of hanging-drop determinations.

DISCUSSION

When the results obtained in semi-solid agar are compared with those of the usual hanging-drop technique, it is evident that the production of a diffuse zone of growth around the line of inoculation in the semi-solid agar serves very satisfactorily to indicate bacterial motility. After incubation for one day in semi-solid agar, the results agreed with those of the hanging-drop method for 99.2 per cent of the cultures. Ultimately, every culture which was motile in hanging-drop preparations was motile also in the semi-solid agar. In the case of 22 strains of the *Escherichia-Aerobacter* group, however, it required two days to elicit a positive reaction by the semi-solid agar method. On the other hand, most of the positive reactions were evident after from 8 to 16 hours. The fact that 20 strains of the *Escherichia-Aerobacter* group, and one spore-forming culture were definitely motile in semi-solid agar, but not in hanging-drop preparation, shows that, in certain instances, the former method will indicate motility when the latter does not. The isolation of motile and non-motile strains from nodular outgrowths and from the non-diffused stab, respectively, is considered to be evidence that only a few cells of the parent culture were motile or potentially motile. Thus, the use of stab cultures in semi-solid agar is distinctly advantageous, when only a small proportion of motile cells is present in a culture, for these may be missed entirely either in hanging-drop preparations or when the semi-solid medium is used in poured plates. The advantages of the semi-solid agar method are particularly evident in teaching schedules and routine testing, because the results are cumulative and macroscopic.

SUMMARY

1. A study of semi-solid agar (nutrient broth plus 0.5 per cent agar) for detecting bacterial motility has been made with 1,051

cultures, representing more than 60 species, and the results obtained compared with those of the usual hanging-drop technique.

2. In semi-solid agar, inoculated by the stab method, motility is manifested macroscopically by a diffuse zone of growth which spreads from the line of inoculation.

3. After incubation for one day, the semi-solid agar method gave results essentially identical with those of the hanging-drop, but after two days it showed 4 per cent more positive cultures.

4. The semi-solid agar method permitted the isolation of motile and non-motile strains from some cultures which were non-motile according to the hanging-drop technique.

5. The semi-solid agar method is particularly advantageous in teaching schedules and routine testing, because the results are cumulative and macroscopic.

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