A comparison of brain heart infusion blood agar sterilized by filtration and heat on the growth of *Neisseria gonorrhoeae*

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SYNOPSIS The growth of *Neisseria gonorrhoeae* on brain heart infusion blood agar in which the base was sterilized by filtration has been compared with growth on the same medium sterilized by heat. Colonies were larger on the unheated medium, and autoclaving at 115°C or 121°C for 15 minutes was accompanied by a progressive decrease in colony size. Viable counts on the three media showed no difference in end points. Colonies on the unheated medium were usually large enough to be easily recognizable after overnight incubation.

The routine medium used for the isolation of *Neisseria gonorrhoeae* in this laboratory is brain heart infusion agar with 10% horse blood and vancomycin, 3 µg/ml, and colistin, 7.5 µg/ml. This usually gives a good growth of gonococci with colonies about 1-2 mm in diameter after incubation for 48 hours in 10% CO₂, but over a period it was noticed that the colonies were extremely small, which made identification difficult. Enquiries suggested that the basal medium might have been left in the autoclave for an unduly long time. This led to an examination of the effect of the temperature of sterilization and sterilization by filtration on the colony size and density of growth of gonococci.

Material and methods

BRAIN HEART INFUSION AGAR (OXOID CM 375)

Of the dehydrated medium, 14.1 g was suspended in 267 ml of distilled water and left at room temperature for 30 minutes. This basal medium was autoclaved at 121°C for 15 minutes, cooled to 48°C, and 30 ml horse blood (Oxoid SR 50) and 3 ml of mixed antibiotic solution containing equal parts of vancomycin 0.06% and colistin 0.15% were added to give final concentrations of vancomycin, 3 µg/ml, and colistin, 7.5 µg/ml. After thorough mixing plates were poured. This medium is designated BH 121/15.

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UNHEATED BRAIN HEART INFUSION AGAR

Double strength brain heart infusion (Oxoid CM 225) was prepared by dissolving 74 g in one litre of distilled water, sterilized by seitz filtration, and stored at 4°C. For use, 150 ml of this base was warmed to 48°C and mixed with 117 ml of 2.5% melted Oxoid Agar No. 1 cooled to the same temperature. Thirty millilitres of horse blood and antibiotics were added as before (BHF medium). For some experiments the filter sterilized base was subsequently sterilized at 115°C or 121°C for 15 minutes. When cooled to 48°C agar, blood and antibiotics were added as before. These media are designated BHF 115/15 and BHF 121/15.

PREPARATION OF INOCULA

These were prepared from:

(a) urethral discharge collected on a swab from males with acute gonococcal urethritis, suspended in 1 ml broth and diluted 1 in 10 in broth;

(b) 24-hour subcultures from primary plates suspended in broth to a turbidity corresponding to between McFarland tubes 1 and 2. Serial 10-fold dilutions in broth were spread on the plates to be compared which were incubated at 36°C in candle jars. In some tests strains which had been subcultured several times were examined. All strains tested were identified as gonococci by their microscopical appearance, the oxidase test, and fermentation reactions.

MEASUREMENT OF COLONIES

This was done on plates which showed discrete colonies. The diameters of 20 or 25 consecutive well separated colonies were measured with a calibrated eyepiece micrometer and a Leitz Ultropak vertical illuminator at a magnification of $\times 22.8$.

Results

The mean diameters of colonies from five primary isolates, 13 subcultures from primary isolates and two stock strains on the filtered medium, filtered and then autoclaved medium and routine medium autoclaved at 121°C for 15 minutes are shown in table I.

Colonies on the filtered medium were consistently larger than those on the medium made from the same constituents but which had been autoclaved. This difference was apparent after incubation for both 24 and 48 hours. Three strains showed no measurable growth when first examined after 24 hours.

In a second experiment, a comparison was made of the colony size of three primary isolates on the filtered medium and on the same medium in which the base had been autoclaved at 115°C or 121°C for 15 minutes (table II).

With these three strains, autoclaving the medium

at 121°C halved the colony size after 24 hours' incubation, and after autoclaving at 115°C the colonies were only three-quarters the size of those on the filtered medium.

These tests showed that medium sterilized by filtration promoted a more rapid growth of gonococci than when the medium was sterilized by heat. To find whether it would promote growth from a smaller inoculum, 0.025 ml volumes of serial dilutions in broth of suspensions of urethral pus from 12 males with acute gonorrhoea were spotted on plates of the two media. When read after 48 hours' incubation there were no significant differences in the end-points. This suggests that although gonococci grew more rapidly on the filtered medium, its sensitivity was not greater than the medium autoclaved at 115°C or 121°C.

To compare the performance of the filtered and autoclaved media under working conditions, 636 urethral swabs from male patients were examined; these were from both treated and untreated patients and were sent to the laboratory on buffered swabs in Stuart's transport medium containing carbon. The order of plating on the two media was alternated. The results are shown in table III.

Strain	Medium			
	BHF	BHF 121/15	BHF	BHF 121/15
	24 h incubation		48 h incubation	
Primary isolates				
G	0.87	0.70	1.40	1.21
G3	0.48	0.24	1.55	0.80
G4	0.69	0.36	2.10	1.48
G5	0.84	0.54	2.02	1.40
G6 ¹	0.20	0.26	1.14	0.98
First subculture from primary				
179	BHF 1.24	BH 121/15 0.91	BHF 2.89	BH 121/15 1.96
312	NG	NG	2.01	0.87
310	1.29	0.85	3.74	2.7
315	1.06	0.81	3.12	2.46
340	0.59	0.40	1.11	1.01
287	1.12	1.01	3.16	2.99
304	1.28	0.83	2.67	1.79
WAK	0.92	NG	2.23	1.08
213	1.26	0.87	3.17	1.25
341	0.49	0.25	1.19	0.57
270	NG	NG	1.97	1.50
319	NG	NG	1.67	1.18
G3a	1.42	1.46	2.04	2.51
Stock strain				
3R	1.12	0.65	—	—
G6 ¹	0.71	0.66	2.56	2.15

Table I Mean diameter (mm) of colonies of gonococci on filtered medium (BHF), filtered and autoclaved medium (BHF 121/15), and medium autoclaved at 121°C for 15 minutes (BH 121/15)

NG = no visible growth.

¹Same isolate.

Medium	Strain					
	No. 1230		No. 1300		No. 1444	
	24 h	48 h	24 h	48 h	24 h	48 h
Filtered	0.56	1.28	0.34	1.26	0.79	1.43
Filtered and autoclaved 115/15	0.46	0.96	0.25	0.92	0.62	1.26
Filtered and autoclaved 121/15	0.27	0.87	0.14	0.64	0.42	0.88

Table II Mean colony diameters (mm) of primary cultures of gonococci on filtered medium and filtered and autoclaved medium

	Filtered medium (BHF)	Autoclaved medium (BH 121/15)
No gonococci grown	558	565
Gonococci grown	78	71
Growth visible after 24 h	67	37
Growth visible only after 48 h	11	34

Table III Routine cultures of urethral swabs from 636 males on filtered and autoclaved media

Two strains were isolated on the autoclaved but not the filtered medium and nine on the filtered but not the autoclaved medium. Growth on the filtered medium was more rapid, and colonies were present in most instances after overnight incubation.

Discussion

The introduction of media made selective for gonococci by the addition of antibiotics by Thayer and Martin (1964) has greatly facilitated the isolation of the organism from contaminated sites. Colonies on selective media are often rather smaller than those on non-selective medium and 48 hours' incubation may be needed before colonies are sufficiently large and characteristic to permit easy recognition. For identification by fluorescent antibody staining (Deacon, 1961) or the staphylococcal co-agglutination test (Danielsson and Kronvall, 1974), young 18-hour colonies are preferable to those grown for 48 hours. For rapid isolation and identification, an ideal medium should permit the development of colonies large enough for recognition after 18 to 24 hours' incubation.

The results summarized in tables I and II suggest that the growth-promoting properties of brain heart infusion base for gonococci are impaired by autoclaving. The filtered medium gave consistently larger colonies than when it had been autoclaved. This was true of both strains which had been subcultured on the autoclaved medium and so might have become acclimatized to it, and primary isolates from urethral pus. As seen in table I, the latter tended to produce smaller colonies than the subcultured strains.

Although medium prepared with filtered brain heart infusion is thought to be preferable, the preparation of large amounts of the sterile filtrate presents problems for most laboratories. Sterilization by autoclaving at 115°C for 15 minutes gave reason-

ably good results although the colonies were rather smaller than those on the filtered medium. Careful control of the autoclaving process is important, and the medium should be exposed to heat for as short a time as possible compatible with sterility being achieved. Cooling is quicker if the medium is distributed in several small containers rather than in one large one. Everall and Morris (1975) autoclaved blood agar base in large and small containers under the same conditions. Colonies of *Streptococcus pyogenes* and *Streptococcus pneumoniae* were smaller when grown on medium from the large container which took a longer time to cool down; viable counts were similar on both batches of medium.

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