INFECTION AND IMMUNITY, Feb. 1977, p. 676-678

Vol. 15, No. 2
Printed in U.S.A.

Improved Minca Medium for the Detection of K99 Antigen in Calf Enterotoxigenic Strains of *Escherichia coli*

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Received for publication 16 August 1976

The K99 antigen of *Escherichia coli* can be detected more readily in cultures grown on Minca medium at 37°C for 6 to 8 h or grown on Minca plus 1% IsoVitaleX for 20 to 24 h.

We reported that K99 antigen was usually undetectable in calf enterotoxigenic *Escherichia coli* cultures grown at 37°C on commercially available nutrient agar plates designed for the isolation of *Enterobacteriaceae*, but easily detectable when grown on a buffered semisynthetic medium at pH 7.5 (Minca). The formulation of Minca is as follows: KH_2PO_4 (Merck), 1.36 g; $Na_2HPO_4 \cdot 2H_2O$ (Merck), 10.1 g; glucose (B.D.H.), 1 g; trace salts solution, 1 ml; Casamino Acids (Difco), 1 g; agar (Difco), 12 g; and distilled water, 1,000 ml. The pH is 7.5. The trace salts solution contained, per liter: $MgSO_4 \cdot 7H_2O$ (Merck), 10 g; $MnCl_2 \cdot 4H_2O$ (Merck), 1 g; $FeCl_3 \cdot 6H_2O$ (Merck), 0.135 g; and $CaCl_2 \cdot 2H_2O$ (Merck), 0.4 g. Some of the strains tested reacted only weakly in K99 antiserum. The detectability was improved when the cultures were subcultured two or three times in liquid Minca. A positive correlation between enterotoxigenicity and the presence of K99 antigen was established (1).

Since then, Minca has been used for the isolation of calf enterotoxigenic *E. coli* strains. It was observed repeatedly that cultures showed strong agglutination in K99 antiserum on primary isolation, but agglutinated only weakly after subculturing on solid Minca. Similar observations were made on strains that were previously shown to possess K99 antigen (1). Improvement was obtained when the agglutinations were done with cultures grown on solid Minca at 37°C for only 6 to 8 h.

K99 antigen was found to possess a fimbria-like morphology (J. S. Teppema, personal communication). It occurred to us that somewhat similar observations were made on *Neisseria gonorrhoeae*. Ninety percent of the primary isolates from acute gonorrhea consisted of type 1 colonies. Upon prolonged incubation (48 to 72 h) or after nonselective in vitro passages, type 3 and 4 colonies originated and eventually predominated (2, 3). Type 1 and 2 colonies possess pili, whereas types 3 and 4 are nonpilated (6, 7). The addition of a defined supplement to the medium was thought to result in a selection of gonococcal type 1 colonies for greater stability (2). IsoVitaleX (BBL; see reference 4) probably has a similar effect.

We presumed that IsoVitaleX might also stabilize the K99 antigen in *E. coli*. In view of this, we reinvestigated the detectability of K99 antigen in 97 enterotoxigenic *E. coli* strains previously found to possess K99 antigen. These strains had been kept on Dorset slopes at room temperature for periods varying from 3 to 8 months. The strains were inoculated onto solid Minca, into liquid Minca (agar omitted), and onto solid Minca without glucose but enriched with 1% IsoVitaleX (Minca-Is). Addition of IsoVitaleX results in the same concentration of glucose as is present in Minca. The presence of K99 antigen in cultures grown on solid media in tubes was investigated by means of the slide

TABLE 1. *Slide agglutination tests with 97 E. coli strains previously shown to possess K99 antigen*

No. of strains	Grown on Minca for:		Passed through liquid Minca (2-3 times) and subcultured onto solid Minca for:		Grown on Minca-Is for:	
	6-8 h	20-24 h	6-8 h	20-24 h	6-8 h	24 h
31	+[a]	+	ND[b]	ND	+	+
11	+	−	+	+	+	+
49	+	−	+	±	+	+
6	−	−	+	± or −	+	+

[a] +, Convincingly positive; ±, not convincingly positive; −, negative.
[b] ND, Not done.

TABLE 2. *Slide agglutination tests with 97 E. coli strains previously shown to possess K99 antigen subdivided per serotype*

Serotype	No. of cultures	Grown on Minca for:		Passed through liquid Minca (2-3 times) and subcultured onto solid Minca for:		Grown on Minca-Is for:	
		6-8 h	20-24 h	6-8 h	20-24 h	6-8 h	20-24 h
O8:K85	15	+[a]	+	ND[b]	ND	+	+
O8:K25	3	+	+	ND	ND	+	+
	11	+	−	+	+	+	+
O20:K?	3	+	+	ND	ND	+	+
O9:K35[c]	3	+	+	ND	ND	+	+
	6	+	−	+	± or −	+	+
	6	−	−	+	± or −	+	+
O101:K28[c]	3	+	+	ND	ND	+	+
	16	+	−	+	± or −	+	+
O101:K30[c]	2	+	+	ND	ND	+	+
	27	+	−	+	+ or ±	+	+
O101:K35[c]	2	+	+	ND	ND	+	+

[a, b] See Table 1.
[c] K antigens of the A variety.

agglutination test after 6 to 8 h of incubation at 37°C in a water bath and after 20 to 24 h of incubation at 37°C. After 20 to 24 h of incubation at 37°C, the cultures in liquid Minca were subcultured into fresh liquid Minca as well as onto solid Minca. This procedure was repeated two or three times. The strains that were passed through liquid Minca and subcultured onto solid Minca were tested for K99 antigen after 6 to 8 h and after 20 to 24 h of incubation at 37°C. All tubes with solid medium were inoculated lightly with a lawn of cells. Six to eight hours of incubation yielded sufficient bacterial growth for use in the slide agglutination test, although individual colonies could not yet be observed.

The results are summarized in Table 1, and a breakdown of these results per serotype is set out in Table 2. All 97 strains were agglutinated convincingly in K99 antiserum when grown on Minca-Is at 37°C for 6 to 8 h as well as for 20 to 24 h. Thirty-one of these strains gave the same results when grown on Minca. An additional 60 strains were agglutinated after 6 to 8 h of incubation, but not after 20 to 24 h of incubation on Minca. Six cultures yielded K99 antigen on Minca only after three passages through liquid Minca (Table 1). All cultures with serotype O8:K85 or O20:K? and 3 of 14 cultures with serotype O8:K25 yielded K99 under all cultural conditions. The other serotypes (having K antigens of the A variety) were mostly cultures that showed K99 antigen only after 6 to 8 h of incubation on Minca.

We surmised that the strains giving negative results under certain cultural conditions did so because the synthesis of K99 antigen was inhibited because of repression of the K99 antigen-synthesizing enzyme(s) by glucose. Perlman and Pastán (5) showed that cyclic 3',5'-adenosine monophosphate overcomes repression by glucose and stimulates many inducible enzymes in *E. coli*. However, we found that neither the addition to Minca of cyclic 3',5'-adenosine monophosphate (1 or 2.5 mM) nor the addition of glycerol, instead of glucose, improved the detectability of K99 antigen after 20 to 24 h of incubation at 37°C.

A possible explanation for the increased detectability of K99 antigen on Minca-Is is that the development of K99 antigen is further improved by the addition of IsoVitaleX. The positive effect of passage through liquid Minca could be explained by assuming that the K99 fimbriae become more stretched or rigid in liquid medium, whereas on solid Minca medium the filaments become increasingly stuck to the bacterial cell surface, particularly after prolonged incubation. The practical implications of the results are obvious. Strains to be tested for K99 antigen should be cultured on Minca-Is or on Minca for only 6 to 8 h at 37°C.

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