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NOTES

Growth of Saprophytic and Pathogenic Leptospira: Evaluation of Medium, Temperature, Inoculum, and Cost

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Of four media tested, a tissue culture medium supplemented with a bovine serum albumin-oleic acid complex provided the best leptospiral growth and cost efficiency.

The growth of high numbers (12×10^7 to 61×10^7/ml) of viable leptospirae in a tissue culture medium (minimum essential medium [MEM]) containing 10% fetal calf serum (FCS) has been reported (4). Replacing the FCS in this medium (MEM-FCS) with bovine serum albumin oleate (MEM-BOH) and vitamin B_{12} has achieved higher yields of viable leptospirae per time of incubation throughout five subcultures. The MEM-BOH medium, when compared with two other serum-free leptospiral media (3, 5), was also found superior. Considering cost, peak cell yield, and time of peak cell yield, the MEM-BOH was found most efficient.

The preparation of Eagle MEM with 10% fetal calf serum MEM-FCS (2), Johnson and Harris (J-H) (5), and Ellinghausen (ELLY) (3) media was done according to the methods described (2, 3, 5). The MEM-BOH was prepared by adding 0.33 ml of 0.43% (vol/vol) sodium oleate (Hormel Institute) to 5 ml of 10% (wt/vol) bovine serum albumin (lipid-poor, fraction V, Miles Laboratories, Inc., Elkhart, Ind.), and then mixing with 4.67 ml of Hanks balanced salts solution (lacking calcium and magnesium salts) (International Scientific Industries, Cary, Ill.) at pH 6.8 and filter sterilizing (0.2-μm pore size membrane filters, Millipore Corp.). A 4-ml volume of this mixture was added to 100 ml of sterile MEM (International Scientific Industries). Vitamin B_{12} was also added to MEM to a final concentration of 0.15 μg/ml.

Pathogenic *Leptospira* serotypes *pomona* Pomona and *canicola* Hond Utrecht IV and saprophytic serotypes *patoc* Patoc I and *biflexa* LT430 were employed in this study. All strains were subcultured monthly in J-H semisolid medium (0.25% Noble agar, Difco Laboratories, Detroit, Mich.) at 25 C and, after two monthly subcultures, transferred once in the respective medium to be studied prior to comparative growth analyses. These cultures were propagated at 30 C, from which final numbers of 10^6 (low inoculum) and 1.5×10^7 (high inoculum) cells of each strain per ml were inoculated (3% vol/vol, final), respectively, into eight quadruplicate sets of tubes, each tube containing 5 ml of medium. Four quadruplicate sets of tubes represented each medium studied, two at each inoculum level at two incubation temperatures (30 and 37 C). All tubes were incubated for 10 days, each serotype being subcultured every 5 days for up to five passages. Viable cell counts were made daily using an agar pour plate method (1). Briefly, a 1:1 mixture of 2×-concentrated respective medium (2.5 ml) and 2% Noble agar (Difco) (2.5 ml) was added to each plate and mixed with the inoculum. Samples of 0.1 ml were taken from each culture, and 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilutions were made in the respective growth medium and plated.

The computed retail cost of ingredients of each medium is 1.1¢/ml of MEM-FCS, 0.87¢/ml of MEM-BOH, 0.4¢/ml of ELLY, and 0.4¢/ml of J-H. Applying this information to the growth data for establishing a base of two, the cheapest cost per cell produced, and associated peak yield time (PYT), a relative cost index (RCI) can be computed for each serotype, medium, and temperature of incubation tested using the following formula: $[O_c/C_c + O_{PYT}/C_{PYT}] - 2 =$ RCI, where C_c = cost of medium ingredients per milliliter divided by peak yield number (PYN) to give cheapest cost per cell, which is established as PYN cost factor base of 1; O_c = cost of ingredients of other media per milliliter divided by PYN to give costs per cell computed not to be the cheapest; C_{PYT} = PYT established as a base of 1 associated with medium computed to give cheapest cost per cell; and O_{PYT} = PYT

associated with medium computed to give not the cheapest cost per cell.

Because of the established base of 2, a formula-derived positive number indicates that level of an inefficient RCI; conversely, a zero or negative number denotes the level of an efficient RCI. Table 1 illustrates the use of the RCI formula.

TABLE 1. *Comparison of MEM-BOH with MEM-FCS at 30 C at an inoculum of 10^6/ml using serotype pomona*[a]

Medium	Cost ($1/ml)	PYN (× 10^7)	Cost/cell ($1/ml × 10^{-12})	PYN cost factor	PYT (days)	PYT factor	RCI
MEM-BOH	0.0087	94	9.26	1.00	4	1.00	0.0
MEM-FCS	0.011	84	13.10	1.41	4	1.00	0.41

[a] For example, 13.10/9.26 + 4/4 = 0.41.

TABLE 2. *PYN, PYT, and RCI of four Leptospira interrogans serotypes when two inoculum levels, four different media, and two incubation temperatures were used*

Serotype	Medium	Temp (C)	Inoculum					
			10^6/ml			1.5 × 10^7/ml		
			PYN (× 10^7)	PYT (days)	RCI	PYN (× 10^7)	PYT (days)	RCI
canicola	MEM-BOH	30	190	3	0.0	175	3	0.0
	MEM-FCS		95	4	1.77	95	4	0.66
	J-H		57	7	1.87	57	2	0.08
	ELLY		49	6	1.79	49	2	0.31
	MEM-BOH	37	192	5	0.0	179	4	0.0
	MEM-FCS		96	3	1.13	98	3	1.16
	J-H		50	6	0.97	59	2	−0.10
	ELLY		46	6	1.12	58	2	−0.08
pomona	MEM-BOH	30	94	4	0.0	92	3	0.0
	MEM-FCS		84	4	0.41	85	4	0.70
	J-H		46	6	0.44	38	2	−0.22
	ELLY		42	6	0.53	30	2	0.08
	MEM-BOH	37	236	5	0.0	211	4	0.0
	MEM-FCS		113	5	1.64	94	3	1.59
	J-H		42	6	1.78	33	2	1.44
	ELLY		40	6	1.91	29	2	1.85
patoc	MEM-BOH	30	270	5	0.0	251	5	0.0
	MEM-FCS		146	4	1.14	153	5	1.07
	J-H		74	6	0.88	74	2	−0.04
	ELLY		70	6	0.97	69	2	0.07
	MEM-BOH	37	266	4	0.0	263	4	0.0
	MEM-FCS		84	3	2.76	85	3	2.66
	J-H		70	6	1.25	66	2	0.33
	ELLY		67	6	1.33	63	2	0.42
biflexa	MEM-BOH	30	282	4	0.0	292	5	0.0
	MEM-FCS		148	4	1.40	147	4	1.31
	J-H		79	6	1.14	65	2	0.46
	ELLY		75	6	1.22	61	2	0.60
	MEM-BOH	37	378	4	0.0	370	3	0.0
	MEM-FCS		274	3	0.49	283	3	0.69
	J-H		77	6	0.76	62	2	1.47
	ELLY		75	6	0.82	79	2	0.87

Table 2 delineates the mean PYN, peak PYT, and associated RCI values of the four serotypes studied.

J-H and ELLY media. Increasing the size of the inoculum (i) shortened PYTs by 4 days (with one exception of 5 days) for all serotypes, regardless of temperature (see Table 3); (ii) significantly decreased PYNs for serotype *pomona* in both media and for serotype *biflexa* in J-H medium only, regardless of temperature; and (iii) increased PYNs for serotype *canicola* in both media at 37 C.

Increasing the temperature of incubation: (i) did not affect PYTs, except for a 1-day decrease for serotype *canicola* with an associated PYN decrease of 12% at the low inoculum level; (ii) at the low inoculum level, significantly decreased PYNs for serotype *pomona* in both media and for serotype *patoc* in J-H medium; and (iii) increased (30%) the PYN for serotype *biflexa* in ELLY medium.

MEM-BOH and MEM-FCS media. Increasing the size of inoculum (Table 3) in both MEM media did not change PYTs consistently or to the same extent as observed in J-H and ELLY media and effected larger PYN and PYT decreases at 37 C for serotype *pomona* in MEM-FCS, but did not change PYNs for the other serotypes at either temperature tested.

Increasing the temperature of incubation caused PYN increases for serotypes *pomona* and *biflexa* in both media, such increases being less for *pomona* but more for *biflexa* in the MEM-FCS medium and, except for *biflexa*, generally smaller at the high inoculum level, and, with one exception, caused PYT decreases at both inoculum levels for all serotypes in MEM-FCS.

In general, increasing the size of inoculum provided more efficient RCI values, except for the MEM-FCS medium and for serotypes *pomona* and *biflexa* at 37 C. Without exception, all serotypes achieved the highest PYNs in the MEM-BOH medium and, with few exceptions, provided the most efficient RCI values. However, the ability to grow other leptospiral serotypes/strains (laboratory subcultures and field isolants) and effects on cell serology and immunogenicity need to be documented for the MEM-BOH medium before it can be recommended for general use.

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TABLE 3. *Summary of the effects of temperature and inoculum size on PYN and PYT of four Leptospira interrogans serotypes*

Culture medium	Culture conditions							
	Inoculum increase				Temp increase			
	30 C		37 C		10^6/ml		1.5×10^7/ml	
	Δ PYN (%)	Δ PYT (days)	Δ PYN (%)	Δ PYT (days)	Δ PYN (%)	Δ PYT (days)	Δ PYN (%)	Δ PYT (days)
MEM-BOH								
canicola				−1		2		1
pomona		−1	11	−1	151	1	129	1
patoc						−1		−1
biflexa		1		−1	34		23	−2
MEM-FCS								
canicola						−1		−1
pomona			−17	−2	34	1	11	−1
patoc		1			−42	−1	−44	−2
biflexa					85	−1	93	−1
J-H								
canicola		−5	18	−4	−12	−1		
pomona	−17	−4	−21	−4			−13	
patoc		−4		−4			−11	
biflexa	−18	−4	−19	−4				
ELLY								
canicola		−4	26	−4			−18	
pomona	−29	−4	−28	−4				
patoc		−4		−4				
biflexa		−4		−4				

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