and found it to be at least several minutes in duration. Other investigators have confirmed this result experimentally (*18*). Parnas and Segal (*19*) showed that it was theoretically possible for a signal of long duration to propagate. The results of Devreotes and Steck (*20*) indicate that the duration of the response depends on that of the stimulus but it can never be longer than several minutes. This suggests that a range of signaling durations might be observed in situ, the longest being several minutes.
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23. There are several models of the temporal relation between the chemotactic movement and signal relay responses. Shaffer (*9*) first proposed that amoebae begin to signal after completing an inward movement step. Other authors have adopted this logical sequence of events (*24*). Cohen and Robertson (*16*) inverted the sequence suggesting that a cell emits a brief cyclic AMP signal about 15 seconds after receiving one and then begins the movement step. This hypothesis has gained acceptance (*4*, *7*) and computer simulations employing this hypothesis lead effectively to aggregation (*25*, *26*).
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27. A plot of the optical density of the fluorograph along the radius of a territory has the contour of a symmetrical wave with at least threefold differences in intensity. We estimated the concentration of the cyclic AMP bands by applying small drops of cyclic AMP directly to the monolayer just before assay. The bands were lighter than the spots produced by drops of $4 \times 10^{-7} M$ cyclic AMP but not as light as those produced by drops of $2 \times 10^{-6} M$. We also conducted control experiments on agar without cells by mixing unlabeled cyclic AMP of known concentration with the ^{3}H-labeled cyclic AMP in the lower filter. The cyclic AMP extracted from cells produced concentrations in the lower filter ranging from $10^{-8} M$ in the interpeak regions to $2 \times 10^{-7} M$ at the peaks. Assuming that extracellular cyclic AMP is a significant fraction of total cyclic AMP (detected in our measurements), the gradients encountered by cells in situ may be as steep as $10^{-6} M$ per millimeter. This is about 50-fold greater than reported threshold values (*28*).
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29. Perhaps the adaptation process (see text) is linked to chemotaxis as well as cyclic AMP signal relay. Cells on the proximal edge of the cyclic AMP wave may be unable to sense the reversed gradient because they are relatively more adapted than those on the distal edge.
30. The duration of movement step is about 100 seconds (*7*, *16*). This is consistent with the dimensions of the cyclic AMP waves revealed by isotope dilution-fluorography. The width of half of the wave (about 0.5 mm) corresponds to a duration of about 90 seconds. A careful examination of movement step durations has revealed a considerable range (60 to 160 seconds) with the mean at 100 seconds (*6*). We predict that the different durations will correlate with differences in the width and velocity of different cyclic AMP waves.
31. A study of the kinetics of the adaptation process yielded detailed information on the increase in cyclic AMP upon introduction of a cyclic AMP stimulus (*32*) and its decrease upon stimulus removal (*33*). This knowledge has been used to sketch the level of the adaptation process within cells in the monolayer. For example, the decay of adaptation occurs with a half-time of about 3 or 4 minutes. In situ, the cyclic AMP wave would advance 0.9 to 1.2 mm in this time.
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35. We thank T. L. Steck for use of his laboratory; T. L. Steck, H. S. Tager, M. C. Dinauer, L. M. Keefer, M. D. Lane, P. Englund, M. Lieber, and M. Logan for helpful discussions and critical review of the manuscript; and M. J. Potel and S. A. Mackay for computer graphical analysis of the fluorograph. The original observation of the thin black lines at the distal edge of the movement bands was made by R. L. Clark. This work was supported by PHS grant GM 22321 to T. L. Steck. P.N.D. was a postdoctoral fellow of the Damon Runyon-Walter Winchell Cancer Fund DRG (178F). The work was also supported in part by PHS grants GM 28007 and RR-5378 to P.N.D.
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Cross-Contamination of Cells in Culture

Abstract. *Lists are presented of references to all known publications describing cell properties that serve to characterize (i) known strains of HeLa and purported human cell lines indicted as HeLa contaminants, (ii) strains of human cell lines contaminated with human but non-HeLa cells, and (iii) strains of cells contaminated by cells from one or more other species. Frequencies of cell cross-contaminations are cited and references are presented to relatively simple techniques that could serve to detect such contamination.*

We present here a comprehensive listing of documented instances of inter- and intraspecies cell culture contamination (Tables 1 to 3) (*1–133*). In 1976 (*26*) we listed the references to all known publications that had served to relate strains of HeLa cells to each other and to indict a large number of other purported human cell lines as HeLa contaminants. A total of 103 sources provided these cultures. Indictment followed when the cells exhibited (i) type A (fast) mobility for G6PD, (ii) type 1 for both PGM1 and PGM3, (iii) absence of a Y chromosome by fluorescent staining, particularly in cases where the cell donors were known to have been male, and (iv) possession of a complex of trypsin-Giemsa banded marker chromosomes reported in known HeLa cells. The list served investigators both as a ready reference to information on HeLa cells and as a signal to the possibility that cells with designations such as those listed might in fact be HeLa cells. The reevaluation of the provenance of published cell lines established from human tumors (*101*) was also of value, because it was among cell lines still extant and available for research that many contaminants were discovered.

When the HeLa cell contamination of many cell lines became known a major effort was made to inform users of cell cultures that in spite of these problems there were available many bona fide lines representing not only the original donors' cells (*102*), but also cells representing the specific tumor of origin (*103*). However, the results of this effort had to be further revised because it was subsequently discovered that while all the cells described were not HeLa, a number of lines had been contaminated with another human tumor cell line, SW-480 (*104*, *105*).

Previously, a number of publications had revealed cell contamination problems, mostly of an interspecies nature, but in general these did not specify the precise contaminating cell line [for summaries see (*106–108*)]. In this report we have tabulated (Table 1) the karyologic, serologic, immunologic, enzymologic, and other data that serve to characterize specific cell cultures. We have updated information on cultures from the same sources as previously listed (*26*) and present references to cultures, many with new designations, from 41 additional sources. The results should serve as an up-to-date reference to contaminated cultures and a further warning that other cultures so designated may be contaminated as well.

Earlier studies concentrated on the mobility patterns of a few isoenzymes (for example, LDH, G6PD, and PGM1 and 2). More recently, cells in culture have been examined for their allozyme genetic signatures representing the composite enzyme phenotype at increasing numbers of loci. The expression of HLA antigens on the cell surface has also been studied more extensively.

In addition to being contaminated with HeLa cells, some human cell lines have been cross-contaminated with other human cells. Detection of type A mobility for G6PD is not in itself sufficient for indictment of a cell as HeLa (*71*, *105*), particularly since there are now a number of newer cell lines expressing this genetic trait (*68*, *71*, *109*). Although this number is still small compared to the number of cells with G6PD type B, it is interesting that a non-HeLa cell with type A may be involved in another contamination event. Thus, one report (*68*) mentions disparity between two cultures of EB-3 cells at different laboratories in regard to G6PD, one being type A, the other type B. EB-3 is a well-known lymphoblast-like cell derived from a patient

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Table 1. Cell lines with characteristics peculiar to HeLa cells. Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; PGM1, phosphoglucomutase, locus 1; ATCC, American Type Culture Collection; CCL, certified cell line number of the ATCC; MBA, Microbiological Associates; GIBCO, Grand Island Biological Company; IMR, Institute for Medical Research; SRI, Stanford Research Institute. Electrophoretic mobility of the following enzymes have also been examined: PGM2 and PGM3, phosphoglucomutase, loci 2 and 3; ESD, esterase D; GLO1, glyoxylase 1; MEM, malic enzyme (mitochondrial); GOTM, glutamic-oxaloacetic transaminase (mitochondrial); ADA, adenosine deaminase; PGD, phosphogluconate dehydrogenase; ACONS, cytosol form of aconitase; PEPA, C, D, peptidases A or C or D; α-GLUC, α-glucosidase; ACP1, acid phosphatase (red cell); AK1, adenylate kinase. The phenotypes of all but four of these enzymes are type 1; the four exceptions are GLO1, type 2; MEM, type 1-2; PGD, type A; and ACP1, type A-B. LDH, lactate dehydrogenase. For references to these enzymes see footnotes a to u. HLA, human lymphocyte antigen was typed in three ways with the following results: 1, negative by cytotoxic reaction (*7*); 2, unstated method gives phenotype A3, A28, BW35 [see (*89*, *97*)]; 3, by a microquantitative absorption technique (*98*) the phenotype for D98 cells was positive for A2 and B12, negative for A1, A3, A9, A10, B5, B7, B8 [see (*99*)]. Other cultures gave identical results except that B12 was negative; in addition, B17 gave a negative reaction (*95*, *100*).

Designation	Reference	Source of cells for study	G6PD type A*	PGM1 type 1	Lack of Y chromosome by banding	Banded marker chromosomes (*1*, *8*)
HeLa (adenocarcinoma, cervix)	*9*, *10*	ATCC[a]	*1*, *5*, *11*, *21*, *22*, *23*, *68*, *69*, *86*	*5*, *68*, *69*, *86*	*1*, *12*, *21*, *22*, *24*, *86*	*1*, *15*, *22*, *24*, *86*
HeLa (=CCL2)		ATCC via A. Daitch			*8*	*8*
		A. Mukerjee			*8*	*8*
		V. Klement from Flow Labs., Inc	*21*		*21*	
		G. Gey			*17*	*17*
		Unlisted[b]	*19*, *90*, *91*, *92*	*19*, *90*, *91*, *92*	*18*	*18*, *19*, *66*, *75*
		Four individuals, unlisted	*4*	*4*		
		GIBCO	*20*	*20*	*20*	*20*
		N. Differante			*64*	*64*
		Flow Labs., Inc.[c]	*68*, *69*	*68*, *69*		
		IMR				*79*
		Johns Hopkins[d]	*89*	*89*†		
$HeLa_1$		Academy of Medicine and Science, Moscow				*83*
$HeLa_2$		Academy of Medicine and Science, Leningrad				*83*
HeLa BU-25	*93*	W. Munyon				*75*
HeLa HB-2-3		Unlisted[e]	*90*	*90*		
HeLa R		Unlisted				*74*
HeLa 229 (=CCL2.1)		ATCC	*23*			
HeLa S_3 (=CCL2.2)		ATCC	*24*		*24*	*24*
HeLa S_3		G. Nette from F. Robbins			*8*	*8*
		Unlisted[f]	*90*	*90*		
		L. Levintow[g] [see (*85*)]	*26*, *86*	*86*	*26*, *86*	*26*, *86*
HeLa S_3g		M. Griffin via G. Melnykovych	*26*		*26*	*26*
HeLa S_3k		K. Kajievara via G. Melnykovych	*26*		*26*	*26*
HCE (carcinoma, cervix)	*47*	R. Brown	*26*		*26*	*26*
LED-Ti (carcinoma, cervix)	*81*	R. Wallace	*82*, *95*			*82*, *95*
JHC (placenta)	*84*	J. Y. Chou	*84*		*84*	*84*
JHT (tumor formed by JHC)	*49*	J. Cho via J. W.-Peng	*84*		*84*	*26*‡, *84*
OE (endometrium)	*50*	The originators [see (*51*)]	*51*			
		P. DiSaia via L. Milewich	*26*		*26*	*26*
AV_3 (amnion)	*25*	Unlisted[h]				
AV_3 (=CCL21)		ATCC[h]	*4*, *5*, *11*, *23*	*4*, *5*	*24*	*15*, *24*
AV_3 (103)		I. Keydar from ATCC	*26*		*26*	*26*
AV_3 (F-49-1)		P. Peebles from ATCC	*26*		*26*	*26*
WISH (amnion)♀	*60*	Individual, unlisted	*4*	*4*		
WISH (=CCL25)		ATCC	*4*, *5*, *11*, *23*	*4*, *5*	*24*	*24*
T-9 (transformed normal diploid, WI38)♀	*42*	J. G. Andzaparidze	*22*		*22*	*22*
AO (amnion)♂	*44*	A. O. Bykovsky	*22*		*22*	*22*
FL (=CCL62) (amnion)	*38*	ATCC	*11*, *23*		*24*	*24*
FLA [probably FL; see (*90*)]		Unlisted[i]	*90*	*90*		
CaOV (carcinoma, ovary)	*39*	N. P. Mazurenko	*22*		*22*	*22*
HBT-3 (carcinoma, breast)	*30*	P. Arnstein from R. Bassin	*1*		*1*	*1*, *71*
G-11 (HBT3 derivative)	*87*	M. Lippman[g]	*86*	*86*	*86*	*86*
HBT-E (16c, clone of HBT-3)		R. Bassin[g]	*1*, *86*	*86*	*1*, *86*	*1*, *86*

Table 1 (continued).

Designation	Reference	Source of cells for study	G6PD type A*	PGM1 type 1	Lack of Y chromosome by banding	Banded marker chromosomes (*1, 8*)
HBT-39b (carcinoma, breast) (clone 6)	*31*	P. Arnstein from E. Plata	*1*		*1*	*1*
BrCA 5 (carcinoma, breast)	*77*	E. Miller	*78*			*78*
ElCo (carcinoma, breast)	*46*	R. Patillo[j]	*26, 69*	*69*	*26*	*26,71*
SH-2 (carcinoma, breast)	*52*	The originators [see (*52*)]	*52*			
		G. Seman via R. Miller				*26‡, 71*
SH-3 (carcinoma, breast)	*52, 73*	The originators [see (*52*)]	*52, 73*			
		G. Seman via R. Miller				*26‡, 71*
BT-20 (carcinoma, breast)	*132*	M. Lippman via R. W. Ruddon¶	*95, 133*		*95, 133*	*95, 133*
Det30A (carcinoma, breast, ascitic fluid)	*11*	W. D. Peterson, Jr.	*11*		*26*	*26*
KB (carcinoma, oral)♂	*14*	Unlisted[k]	*19, 90*	*19, 90*	*19*	*19*
KB (=CCL17)		ATCC[l]	*5, 11, 21, 23, 68, 69*	*5, 68, 69*	*12, 21, 24*	*15, 24*
		S. Mak	*20*	*20*	*20*	*20*
		V. Klement from MBA	*21*		*21*	
		H. Sussman	*26*		*26*	*26*
		E. Priori	*26*		*26*	*26*
		Flow Labs., Inc.[c]	*68, 69*	*68, 69*		*79*
		Commercial, unlisted	*4*	*4*		
H. Ep.-2 (carcinoma, larynx) ♂	*16*	Unlisted[h]	*19*	*19*		
H. Ep.-2 (=CCL23)		ATCC[m]	*5, 11, 21, 23, 68*	*5*	*12, 21, 24*	*24*
		Individual, unlisted	*4*	*4*		
		P. Dent	*20*	*20*	*20*	*20*
		V. Klement from MBA	*21*		*21*	
		M. Webber	*26*		*26*	*26*
		K. McCormick			*64§*	*64*
		Commercial, unlisted				*79*
		Flow Labs., Inc.[c]	*68, 69*	*68, 69*		
		Unlisted[n]	*90*	*90*		
H. Ep.-2 (clone)		K. V. Ilyin	*22*		*22*	*22*
CaVe (carcinoma, stomach)	*67*	Unlisted (two cultures)				*66*
Minnesota EE (esophageal epithelium) ♂	*57*	Individual, unlisted	*4*	*4*		
Minnesota EE (=CCL4)		ATCC	*4, 5, 11, 23*	*5*	*24*	*24*
Intestine 407 (jejunum, ileum) (=CCL6)		ATCC	*5, 23, 24*	*5*	*24*	*24*
Intestine 407	*58*	Commercial, unlisted	*4*	*4*		
Intestine 407 (=HEI=CCL6)		G. Spahn from ATCC	*26*		*26*	*26*
CMP (adenocarcinoma, rectum) ♂	*48*	Unlisted	*5*	*5*		
CMPII C2♂	*48*	D. Rounds via J. Kim	*26*		*26*	*26*
Chang liver (liver)	*29*	Unlisted[h]	*19*	*19*		
Chang liver (=CCL13)		ATCC[j]	*4, 5, 23, 69*	*4, 5, 69*	*24*	*24*
Chang liver (=CLL 74)		R. Chang[g, o]	*26, 86*	*86*	*26, 86*	*26, 86*
Chang liver		F. Deinhardt[p]	*95*	*95*	*95*	*95*
		Individual, unlisted	*4*	*4*		
Det6 (sternal marrow)♂	*56*	Unlisted[h]				
		Commercial	*4*	*4*		
Det6 (=CCL3)		ATCC	*5, 23*	*5*		
Det6 (clone 12) (=CCL3.1)		ATCC	*23*		*24*	*24*
Det6 (=CCL3)		Child Research Center of Michigan or ATCC [see (*12*)]	*11*		*12*	
Detroit-6		Academy of Medicine and Science, Leningrad				*83*
Detroit 98 (=CCL18) (sternal marrow)♂	*23, 37*	ATCC	*4, 5, 23*	*4, 5*	*24*	*24*
		Unlisted[q]	*90*	*90*		
Detroit 98s (=CCL18.1)	*23*	ATCC	*23*		*24*	*24*
Detroit 98/AG (=CCL18.2)	*23*	ATCC	*23*		*8, 24*	*8, 24*
Detroit 98/AH-2 (=CCL18.3)	*23*	ATCC	*23*		*13, 24*	*13, 24*
		B. O. Bengtsson				*79*

Table 1 (continued).

Designation	Reference	Source of cells for study	G6PD type A*	PGM1 type 1	Lack of Y chromosome by banding	Banded marker chromosomes (*1, 8*)
Detroit 98/AHR (=CCL18.4)	*23*	ATCC	*23*		*24*	*24*
J96 (leukemic blood)♀	*40*	T. A. Bektemirov	*22*		*22*	*22*
J111 (monocytic leukemia)♀ (C=CCL24)	*41*	Commercial, unlisted	*4*	*4*		
		Flow Labs., Inc.[c]	*68, 69*	*68, 69*		
		Unlisted[h]				
		ATCC	*5, 23, 68*	*5*	*24*	*24, 72*
ESP_1 (Burkitt lymphoma, American)♂	*53*	P. Price from E. Priori	*26*		*26*	*26*
		E. Priori	*26*		*26*	*26*
L-132 (=CCL5) (lung)	*28*	ATCC	*5, 23*	*5*	*24*	*24*
L-132 (G-38-7)		P. Peebles from ATCC	*26*		*26*	*26*
LU (fetal lung)	*94*	Unlisted[r]	*90*	*90*		
LU 106 (embryonic lung)♂	*80*	Unlisted				*79*
2563 (=MAC-21) (carcinoma, lung)♂	*88*	R. Akeson[g]	*86*	*86*	*86*	*86*
HuK°39 (kidney)		M. J. Colston	*95*		*95*	*95*
T-1 (kidney)♂	*70*	Five separate cultures: J. van der Veen, G. W. Barendsen, P. Todd, E. A. Blakely, M. R. Raju[s]	*100*	*100*	*100*	*100*
HEK (kidney)	*32*	Commercial, unlisted	*4*	*4*		
		J. Rhim from C. Pfizer Inc.	*1*		*1*	*1*
		C. Pfizer, Inc.	*1*		*1*	*1*
HEK/HRV (HEK, virus transformed)	*33*	S. Aaronson	*26*		*1*	*26*
RT4 (carcinoma, bladder) ♂	*36*	J. Leighton via N. Abaza¶	*1*		*1*	*1*
MA 160 (prostate)	*34*	The originators [see (*34*)]			*34#*	
		P. Price, MBA[g]	*1, 86*	*86*	*1, 86*	*1, 86*
		M. Vincent, MBA	*12*		*12*	*27*
Prostate (=MA160)		Unlisted	*5*	*5*		
KP-P_1 (carcinoma, prostate)	*45*	P. Lee via M. Glovsky	*26*		*26*	*26*
EB33 (carcinoma, prostate)	*54*	F. Schroeder	*26*		*26*	*26*
D18T (synovial cell)	*55*	D. A. Peterson	*26*		*26*	*26*
M10T (synovial cell)	*55*	D. A. Peterson	*26*		*26*	*26*
SA4 (TxS-$HuSa_1$) (liposarcoma)♂	*35*	C. Pfizer, Inc.	*1*		*1*	*1*
SA4 ♂		D. Morton[m]				
DAPT (astrocytoma, piloid) ♂	*43*	A. O. Bykovsky	*22*		*22*	*22*
Girardi heart (=CCL27) (heart)♂	*61*	ATCC[h]	*4, 5, 23*	*4, 5*	*24*	*24*
TuWi (=CCL31)	*62*	ATCC	*23*		*24*	*24*
Wong-Kilbourne (=CCL20.2) (conjunctiva)	*63*	ATCC	*23*		*24*	*24*
Hut (tissue not specified)		Neither reference nor source listed; [see (*69*)][t]	*69*	*69*		
F255A4 (tissue not specified)		Neither reference nor source listed; [see (*69*)][t]	*69*	*69*		
EUE (fetal subcutis)	*96*	Unlisted[u]	*90*	*90*		
NCTC2544 (=CCL19) (skin epithelium)♂	*59*	ATCC	*4, 5, 11, 23*	*4, 5*	*12, 24*	*24*
NCTC3075 (=CCL19.1)♂	*59*	ATCC	*23*		*24*	*24*

*Type A mobility for G6PD has been shown for all cultures in this list. However, to our knowledge, type B was reported only once (*3*), for cultures designated Detroit 6, Detroit 6 (clone 12), and Chang liver, all obtained from the ATCC. †Locus not indicated, presumably PGM1. ‡Karyotypes of banded chromosomes sent to us by the investigator (source) for study. §As discussed in (*65*), the work of Sinha and Pathak (*64*) on HeLa and H.Ep.-2 cells does not make it clear whether the authors consider these cells to be of separate origin or both derived from HeLa. Nevertheless, this work is unique in that 4.5 percent of H.Ep.-2 cells exhibited a Y chromosome. No other record of presence of Y is known in the cells except as mentioned for MA160 [see (*34*) and footnote #]. ‖F. Kasten, personal communication (1975), indicated that CMP had been derived from adenocarcinoma of the colon. ¶As indicated (*65*), bona fide BT-20 cells exist; the same is true for RT4 cells (*2*). #The original publication stated that a Y chromosome was observed in cells at passage 10, but was not seen banded (*12, 27*) at earlier or later passages. [a]For PGM3, ESD, GLO1, ADA, PGD, and PEPD, see (*68, 69, 86*). HLA results by method number 1, see (*7*). [b]For all enzymes except AK1, see (*6, 90–92*). HLA results by method number 2, see (*97*). [c]For PGM3, ESD, GLO1, ADA, PGD, and PEPD, see (*68, 69*). [d]For ADA, PGD, ACP1, AK1, and HLA results by method number 2, see (*89*). [e]For all enzymes except GLO1, MEM, ACONS, PEPD, and α-GLUC, see (*90*). [f]For all enzymes except ESD, GLO1, ACONS, PEPD, α-GLUC, and AK1, see (*90*). [g]For PGM3, ESD, GLO1, ADA, PGD, and PEPD, see (*86*). [h]For PGM3, see (*6*). [i]For PGM2, PGM3, and GOTM, see (*90*). [j]The same enzymes as in footnote c, see only (*69*). [k]The same enzymes as in footnotes e and h, see (*6, 90*). [l]The same enzymes and HLA results as in footnote a, but only see (*7, 68, 69*). [m]HLA results by method number 1, see (*7*). [n]For enzymes PGM2, PGM3, ESD, GOTM, PEPA, and PEPC, see (*90*). [o]HLA results by method number 3, see (*95*). [p]The same enzymes as in footnote g, see (*95*), and HLA as in footnote o. [q]For all enzymes except MEM, PEPD, α-GLUC, and AK1, see (*90*). Eleven enzyme phenotypes in addition to those cited here were examined for a culture of D98 (=Detroit 98), see (*90*). [r]For all enzymes except ESD, GLO1, MEM, ACONS, PEPD, α-GLUC, and ACP1, see (*90*). [s]Same enzymes as in footnote g, see (*100*), and HLA as in footnote o, see (*100*). [t]For PGM3, ESD, GLO1, ADA, and PEPD, see (*69*). [u]For all enzymes except ESD, GLO1, MEM, ACONS, PEPD, α-GLUC, and ACP1, see (*90*).

Table 2. Non-HeLa, intrahuman cell line contamination.

Designation	Reference	Donor or tissue characteristic	Actual characteristic	Methods of determination
TDL-1	*110*	Non-neoplastic tonsils, lymphoid, ♂	P3JHR-1, Burkitt lymphoma, ♂ (*111*)	Chromosome banding, HLA antigen typing, growth characteristics, tumorigenicity, expression of Epstein-Barr virus antigen and surface membrane immunoglobulins (*112*)
TDL-2		Same as above, ♀	Same	
TDL-3		Same as above, ♂	RPMI 1788, lymphoblastoid cell, normal donor, ♂	
TDL-4		Same as above, ♀	Raji, Burkitt lymphoma, ♂ (*113*)	
Raji	*113*	PGD type unknown	PGD types A and C recorded	Isoenzyme electrophoresis (*68*)*
EB-3	*114*	G6PD type unknown	G6PD types A and B recorded	Isoenzyme electrophoresis (*68*)*
Hs852 Hs852L	*115*	PGM3 and ESD types unknown	PGM3 types 2 and 1–2 and ESD types 1 and 1–2, respectively, at two different passage levels	Isoenzyme electrophoresis (*68*)
SW-527 SW-598 SW-608 SW-613 SW-732 SW-733	*105*	Individually derived from six different tumors in six different patients	All identical to two separate tumor lines (SW-480 and SW-620) from colon adenocarcinoma of the same individual, ♂ (*105*)†	Chromosome banding and isoenzyme electrophoresis (*104, 105*)†
RY	*129*	Hodgkin's disease spleen cells	Neither the patient nor the cells exhibit Hodgkin's disease or tumor cell or macrophage characteristics (*131*)	Indistinct histogenesis, lack of surface receptors of macrophages, lack of lysozyme production (*131*)
A series of cultures	*71* (see M. Fraccaro in discussion)	Mammary carcinoma	All cultures the same, donor unknown. G6PD "different from A and B"	Chromosome banding, enzyme electrophoresis

*O'Brien *et al.* (*68*) state that their stock of Raji exhibited PGD type C mobility, whereas a culture was scored as A by H. Harris, and that their stock of EB-3, another Burkitt lymphoma line, was G6PD-A, while that of Harris was G6PD-B. This indicates a likely contamination problem involving these two cell lines. The donors' phenotypes are unknown. †C- and Q-banded Y-like chromosomes were noted in 4 to 5 percent of the SW-480 cells, but not in any other culture (*105*).

Table 3. Interspecies cell line contamination.

Designation	Reference	Source		Method of determination
		Purported	Actual	
GPS-PD♂ and GSP-M	*106*	Guinea pig spleen, adult	Mouse, L-M strain of L cells (*109*)	Serologic and karyologic (conventional staining) (*106*)
Suitor's clone of *Aedes aegypti*	*116*	Mosquito	Moth, Grace's *Antheraea eucalypti* (*117*)	Immunologic, karyologic and isoenzyme electrophoresis (*116*)
Culisita inornata		Mosquito	Same as above	
Aedes vexans		Mosquito	Same as above	
LT-1	*118*	Grass frog renal adenocarcinoma	Contaminated with two different cells: TH, box turtle heart (*119*) and FHM, fat head minnow (*120*)	Chromosome analysis, isoenzyme electrophoresis (*121*)
CHB	*122*	Human, astrocytoma	Rat, has some characteristics of glial cells; not C-6 strain (*123*)	Chromosome analysis, isoenzyme electrophoresis (*124*)
HBC	*125*	Human, invasive duct cell carcinoma, breast	Rat, altered	Chromosome banding, isoenymze electrophoresis, immunofluorescence (*78*)
HEL-R66	*126*	Human	Monkey, *Cercopithecus aethiops* (*127*)	Chromosome banding, isoenzyme electrophoresis, immunofluorescence (*128*)
FQ SpR RB	*129*	Human, Hodgkin's spleen cells	Owl monkey, *Aotus trivirgatus*, kidney cell line, OMK-210 (*130*)	Chromosome banding, isoenzyme electrophoresis (*131*)
CaMa (clone 15)	*62, 67*	Human, carcinoma, breast	Syrian hamster, *Mesocricetus auratus* (*95*)*	Chromosome banding isoenzyme electrophoresis, immunofluorescence (*95*)
McCoy (1968)	See note added in proof; S. M. McConnell	Human	Mouse, strain L	Conventional karyology (*95*)
McCoy (1981)	R. W. Eimmons from J. Schacter	Human	Mouse, strain L†	Chromosone analysis, immunofluorescence (*95*)
McCoy's RA (1981)	P. Price, from Dr. Wong, from D. Alexander	Human	Mouse, strain L	Chromosome analysis, immunofluorescence (*95*)

*CaMa, not to be mistaken with Cama 1 (*102*) has been suspected by us of being HeLa. Cells of the original culture are not available. In the present situation a "parental" culture was thought to be that of Syrian hamster whereas two substrains were clearly of murine origin as shown by chromosome and isoenzyme results [S. Povey in communication to M. Green (*67*)]. †N. J. Schmidt indicated that while these cells are said to be human they are positive for murine cells by fluorescent antibody tests (*139*).

with Burkitt's lymphoma. The stock of EB-3 (CCL85) of the ATCC is type A (*109*), the donor's genotype, however, is not known.

In Table 2 we list this and other examples of non-HeLa cell contamination of human cells. The G6PD pattern for the TDL cells is not known, but results of techniques such as HLA-A and -B phenotyping, detection of the presence or absence of Epstein-Barr virus (EBV) antigens, and distinction of surface membrane immunoglobulins serve to identify and thus detect cross-contamination involving lymphoid cells. There are a few reports of cross-contamination involving the type B phenotype for G6PD (*68, 105*): for example, in one instance a discrepancy was detected between an early- and late-passage culture of a melanoma-derived line Hs852, and in another case six independently derived lines initiated from solid tumors were found to be identical to each other, probably because they were contaminated by an additional line started in the same laboratory.

In Table 3 we summarize a number of interspecies contaminations, providing specific information on the characteristics of the particular cells. Although most documented cases of contamination involve entire cultures with the same contaminating cells, instances of mixed-cell populations persisting in a stock have been reported (*134*). We cite two examples. In one, grass frog cells (LT-1) were supplanted by box turtle heart (TH) cells and fat head minnow (FHM) cells. In the second, which involved a mishap in production of stock cultures (not listed in Table 3), a male muntjak cell line was contaminated by rat cells and the two lines continued to grow side by side. In this instance the karyotypic differences were so pronounced that further testing was unnecessary [see (*71*, figure 36)].

We have cited only major instances of cell cross-contamination, but it must be emphasized that such events occur quite frequently. Undoubtedly many cases go unnoticed or are detected instantly, and corrected, in the course of experimentation. The frequency of intra- and interspecies contamination events can be surmised from four publications that offer résumés of experiences with cultures submitted specifically for monitoring tests (*69, 71, 78, 134*).

The risk of contamination or overgrowth of cultures by unrelated cells is a potential and often recurring problem where cells are grown and studied. With relatively simple techniques, however, and a continuing program of monitoring for cell line purity, one can be assured of working with bona fide cell cultures.

Note added in proof: In 1968 we examined a culture designated "McCoy," presumably derived from human synovial cells (*135*), and determined that the cells were of mouse origin and probably strain "L" (Table 3). This confirmed an earlier report (*136*). No known reference exists to either the initiation or originator of this culture, and while it is believed to have been initiated by C. M. Pomerat, who perhaps grew the cells on McCoy's medium, it was *not* initiated by T. McCoy (*137*). These cells, often thought to be human in origin, are being widely distributed, in part because they are used for propagating the pathogen *Chlamydia trachomatis* (Table 3). It is suggested that bona fide "L" cells of known murine origin be used for this purpose because they grow the pathogen equally well (*138*) and would not perpetuate the dispersal of unknown cell substrates.

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Epstein-Barr Viral DNA: Infectivity for Human Placental Cells

Abstract. *Purified DNA of Epstein-Barr virus (EBV) is regularly infectious by means of the "calcium" method of transfection. Cultured human placental cells exposed to EBV DNA of two transforming strains, FF41 and B95, produce virus that is capable of converting normal B lymphocytes into established cell lines. After treatment with EBV (FF41) DNA and EBV (HR-1) DNA the placental cells display antigens associated with the productive viral cycle. The placental cells have not developed foci or other signs of morphologic transformation.*

Epstein-Barr virus (EBV), a human lymphotropic herpesvirus, is the cause of infectious mononucleosis and has been implicated in the etiology of three human cancers, nasopharyngeal carcinoma, Burkitt lymphoma, and immunoblastic lymphoma. The biologic behavior of the virus has been studied mainly in lymphocytes. Nearly all strains of EBV are capable of converting normal B lymphocytes of man and certain species of nonhuman primates into continuous (immortalized) cell lines (*1*). One exceptional nontransforming strain of the virus, called P3HR-1, undergoes an abortive cycle of replication in certain established lymphoblastoid cell lines of B cell origin (*2*, *2a*). Since only a small fraction of transformed human B lymphocytes permits viral replication, and since there is no fully permissive cell, it has been difficult to analyze the viral replicative cycle or to derive viral mutants. Until now the principal way to propagate the virus was to "immortalize" marmoset cells, a process that requires several weeks to months. Virus can then be harvested from supernatant fluids of continuous marmoset lymphoid cells which are more permissive of viral replication than comparable human cells (*3*, *4*).

The tropism of EBV for lymphocytes is apparently related at least in part to a virus "receptor" present on B cells (*5*). The host range of EBV can be enlarged if the usual mechanism of entry by the virus is bypassed (*6*). Microinjection of the DNA of the cytolytic EBV variant, P3HR-1, leads to viral "early antigen" expression in human and rat fibroblasts and monkey kidney cells. Implantation of B lymphocyte membranes by the process of membrane fusion has recently been shown to allow EBV to enter murine cells and T lymphocytes; the genome is expressed in such cells because they contain viral encoded antigens (*6*). Thus far, the only nonlymphoid cells in which infectious EBV has been propagated in the laboratory are malignant epithelial cells of nasopharyngeal carcinoma that have been passaged in nude mice (*4*). There has been no evidence that mature virus will replicate in a monolayer culture of uninfected normal fibroblastic cells, or that the viral DNA is infectious when conventional transfection methods are used.

For the experiments described here we chose human placental cells because we had found (*7*) that they were susceptible to transfection with the DNA's of

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