HeLa Cultures Defined

Abstract. *A list is presented of references to all known publications on properties which have served to relate strains of HeLa cells to each other as well as to indict other purported human cell lines as HeLa cell contaminants. Eleven additional cell lines not previously indicted are described. When they exhibit (i) type A (fast) mobility for glucose-6-phosphate dehydrogenase, (ii) phosphoglucomutase type 1 at locus 1 and locus 3, (iii) absence of a Y chromosome by fluorescent staining, and (iv) possession of a complex of trypsin-Giemsa banded marker chromosomes present in known HeLa cells, then cell substrates regardless of designation should be considered de facto strains of HeLa.*

Since our report last year (*1*) about HeLa cell contamination among some human tumor cells, we have examined many cultures sent to our laboratory by investigators in the United States and abroad who were interested in knowing whether their cells had the HeLa cell characteristics that we discussed. Many did turn out to have them.

In addition, most of these investigators, as well as other members of the scientific community, expressed the desire to have clearly publicized an up-to-date list of all "cell lines" which by various methods, but particularly by newer techniques, have been indicted as HeLa cell contaminants (that is, they exhibit type A mobility for glucose-6-phosphate dehydrogenase, lack a Y chromosome, have, in addition to normal chromosomes, a complex of rearranged chromosomes or markers described for HeLa cell cultures).

We have assembled and tabulated all information available and published relating to this matter (Table 1) and have added to the list those newer "cell lines" examined by us (*26*) which we consider to be of HeLa origin. We have not added earlier records which indicated only that some of these cell cultures were of human (or primate) origin and not of other animal origin on the basis of their susceptibility to poliovirus nor have we compiled serologic or immunologic data which only defines the species of origin. We have added, where appropriate, the important information, largely ignored, on the genetically determined phosphoglucomutase activity of HeLa cells (*4–6, 19, 20*) as well as some data on HL-A antigen reactivity (*7*) of these particular human cells.

The source of each culture is given, when known, because as indicated by Franks and Rigby (*2*) and one of us, it is possible that there do exist bona fide cell cultures

Table 1. Cell lines with characteristics peculiar to HeLa cells including previously published data and more recent results by newer techniques. Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; ATCC, American Type Culture Collection; MBA, Microbiological Associates.

Designation	Reference	Source	G6PD type A*	PGM†		Lack of Y chromosome by banding	Banded marker chromosomes (*1,8*)
				Type 1	Type 3		
HeLa (adenocarcinoma cervix)‡	*9,10*	ATCC	*1,5,11,21, 22,23*	*5*		*1,12,21,22,24*	*1,15,22,24*
HeLa (=CCL2)§		ATCC via A. Deitch				*8*	*8*
		A. Mukerjee				*8*	*8*
		V. Klement from Flow Labs., Inc.	*21*			*21*	
		G. Gey				*17*	*17*
		Unlisted	*19*	*19*	*6*	*18*	*18,19*
		Four individuals, unlisted	*4*	*4*			
		Grand Island Biological Co.	*20*	*20*		*20*	*20*
		N. Differante				*64*	*64*
HeLa 229 (=CCL2.1)		ATCC	*23*				
HeLa S_3		G. Nette from E. Robbins				*8*	*8*
		L. Levintow	*26*			*26*	*26*
HeLa S_3g		M. Griffin via G. Melnykovych	*26*			*26*	*26*
HeLa S_3k		K. Kajievara via G. Melnykovych	*26*			*26*	*26*
KB (carcinoma, oral)	*14*	Unlisted	*19*	*19*	*6*	*19*	*19*
KB (=CCL17)‡		ATCC	*5,11,21,23*	*5*		*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*
		Commercial, unlisted	*4*	*4*			
H.Ep.-2 (carcinoma, larynx)	*16*	Unlisted	*19*	*19*	*6*		
H.Ep.-2(=CCL23)‡		ATCC	*5,11,21,23*	*5*		*12,21*	
		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*
H.Ep.-2 (clone)		K. V. Ilyin	*22*			*22*	*22*
AV3 (amnion)	*25*	Unlisted			*6*		
AV3 (=CCL21)		ATCC	*4,5,11,23*	*4,5*	*6*	*24*	*15,24*
AV3 (103)		I. Keydar from ATCC	*26*			*26*	*26*
AV3 (F-49-1)		P. Peebles from ATCC	*26*			*26*	*26*
L132 (=CCL5) (lung)	*28*	ATCC	*5,23*	*5*		*24*	*24*
L132 (G-38-7)		P. Peebles from ATCC	*26*			*26*	*26*
Chang liver (liver)	*29*	Unlisted	*19*	*19*	*6*		
Chang liver (=CCL13)		ATCC	*4,5,23*	*4,5*		*24*	*24*
		R. Chang	*26*			*26*	*26*
		Individual, unlisted	*4*	*4*			
HBT3 (carcinoma, breast)	*30*	P. Arnstein from R. Bassin	*1*			*1*	*1*
HBT-E (16c, clone of HBT-3)		R. Bassin	*1*			*1*	*1*
HBT-39b (carcinoma, breast) (clone 6)	*31*	P. Arnstein from E. Plata	*1*			*1*	*1*

Designation	Reference	Source	G6PD type A*	PGM‡ Type 1	PGM‡ Type 3	Lack of Y chromosome by banding	Banded marker chromosomes (*1,8*)
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*
MA160 (prostate)	*34*	The originators (see *34*)				*34*¶	
MA160		P. Price, MBA	*1*			*1*	*1*
		M. Vincent, MBA	*12*			*12*	*27*
Prostate (=MA160)		Unlisted	*5*	*5*			
SA4(TxS-HuSa$_1$) (liposarcoma)	*35*	C. Pfizer, Inc.	*1*			*1*	*1*
SA4‡		D. Morton					
RT4 (carcinoma, bladder)	*2,36*	J. Leighton via N. Abaza**	*1*			*1*	*1*
Detroit 30A (carinoma, ascitic fluid)	*11*	W. D. Peterson, Jr.	*11*			*26*	*26*
Detroit 98 (=CCL18) (sternal marrow)	*23,37*	ATCC	*4,5,23*	*4,5*		*24*	*24*
Detroit 98s (=CCL18.1)	*23*	ATCC	*23*				
Detroit 98/AG (=CCL18.2)	*23*	ATCC	*23*			*8*	*8*
Detroit 98/AT-2 (=CCL18.3)	*23*	ATCC	*23*			*13*	*13*
Detroit 98/AHR (=CCL18.4)	*23*	ATCC	*23*				
FL (=CCL62) (amnion)	*38*	ATCC	*11,23*			*24*	*24*
CaOV (carcinoma, ovary)	*39*	N. P. Mazurenko	*22*			*22*	*22*
J96 (leukemic blood)	*40*	T. A. Bektemirov	*22*			*22*	*22*
J111 (monocytic leukemia) (=CCL24)	*41*	Commercial, unlisted	*4*	*4*			
		Unlisted			*6*		
		ATCC	*5,23*	*5*			
T-9 (transformed normal diploid)	*42*	O. G. Andzaparidze	*22*			*22*	*22*
DAPT (astrocytoma, piloid)	*43*	A. O. Bykovsky	*22*			*22*	*22*
AO (amnion)	*44*	A. O. Bykovsky	*22*			*22*	*22*
KP-P$_1$ (carcinoma, prostate)	*45*	P. Lee via M. Glovsky	*26*			*26*	*26*
ElCo (carcinoma, breast)	*46*	R. Patillo	*26*			*26*	*26*
HCE (carcinoma, cervix)	*47*	D. Brown	*26*			*26*	*26*
CMP (adenocarcinoma, rectum)††	*48*	Unlisted	*5*	*5*			
CMPII C2	*48*	D. Rounds via J. Kim	*26*			*26*	*26*
JHT (placenta)	*49*	J. Cho via J. W.-Peng					*26*‡‡
OE (endometrium)	*50*	The originators (see *51*)	*51*				
		P. Di Saia via L. Milewich	*26*			*26*	*26*
SH-2 (carcinoma, breast)	*52*	The originators (see *52*)	*52*				
		G. Seman via R. Miller					*26*‡‡
SH-3 (carcinoma, breast)	*52*	The originators (see *52*)	*52*				
		G. Seman via R. Miller					*26*‡‡
ESP$_1$ (Burkitt lymphoma, American)	*53*	P. Price, from E. Priori	*26*			*26*	*26*
		E. Priori	*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*
Detroit 6 (sternal marrow)	*56*	Unlisted			*6*		
		Commercial, unlisted	*4*	*4*			
Detroit 6 (=CCL3)		ATCC	*5,23*	*5*		*24*	*24*
Detroit 6 (clone 12) (=CCL3.1)		ATCC	*23*				
Detroit 6 (=CCL3)		Child Research Center of Michigan or ATCC (see *12*)	*11*			*12*	
Minneosta 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*	*5*			
Intestine 407	*58*	Commercial, unlisted	*4*	*4*			
Intestine 407 (=HEI=CCL6)		G. Spahn from ATCC	*26*			*26*	*26*
NCTC2544 (=CCL19)(skin) (epithelium)	*59*	ATCC	*4,5,11,23*	*4,5*		*12,24*	*24*
NCTC3075 (=CCL19.1)	*59*	ATCC	*23*				
WISH (amnion)	*60*	Individual, unlisted	*4*	*4*			
WISH (=CCL25)		ATCC	*4,5,11,23*	*4,5*		*24*	*24*
Girardi heart (heart)(=CCL27)	*61*	ATCC	*4,5,23*	*4,5*	*6*		
TuWi (=CCL31)	*62*	ATCC	*23*				
Wong-Kilbourne (conjunctiva) (=CCL20.2)	*63*	ATCC	*23*				

*Type B mobility for glucose-6-phosphate dehydrogenase has been reported, to our knowledge, only once (*3*) for cells in this list, and that for cultures designated Detroit 6, Detroit 6 (clone 12), and Chang liver, all obtained from the ATCC. Otherwise, all of these cultures have exhibited type A mobility. †Phosphoglucomutase electrophoretic variants determined by alleles at locus PGM$_1$ (=type $\frac{1}{1}$) and the separate, unlinked locus PGM$_3$ (type $\frac{3}{3}$) (*4–6,9,19,20*). ‡Like HeLa, no reactivity when tested by the human lymphocyte antigen cytotoxic reaction (*7*). §CCL = certified cell line number of the ATCC. ||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 whether they are 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 these cells except as mentioned for MA160 [(*34*) and ¶]. ¶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. **As indicated (*2*), bona fide RT4 cells exist. ††F. Kasten, personal communication (1975) indicated that CMP had been derived from adenocarcinoma of the colon. ‡‡Karyotypes of banded chromosomes sent to us by the investigator (Source) for study.

with their proper designations as well as HeLa contaminants which masquerade under the same name.

It is, no doubt, true that the different bona fide strains of HeLa perform in different ways and exhibit many distinct characteristics; the same is true of cultures of HeLa that are known by different designations, but are de facto HeLa strains themselves. Nevertheless, these strains have retained the characteristics that we have tabulated here, in spite of different passage levels under different growth conditions and in different laboratories. It is this group which we specifically addressed here and the one which has led many investigators to believe that "HeLa by many other names can spell trouble."

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Phytoestrogens: Adverse Effects on Reproduction in California Quail

Abstract. *Phytoestrogens, largely formononetin and genistein, are produced in the leaves of stunted desert annuals in a dry year. When ingested by California quail, these compounds apparently inhibit reproduction and prevent the production of young that will not have adequate food. In a wet year, forbs grow vigorously and phytoestrogenic substances are largely absent. Quail then breed prolifically and the abundant seed crop carries the enlarged population through the winter.*

The California quail, *Lophortyx californicus*, breeds irregularly in the more arid portions of its range, depending on the amount of winter rainfall preceding the spring nesting season. When rainfall is generous, a rich carpet of forbs which supplies both greens and seeds for quail consumption is produced. Under this circumstance the birds breed vigorously. In a relatively dry year, the ground may be sparsely covered with stunted forbs and annual grasses; then quail breeding is desultory, and few or no young are produced. There seems to be some direct connection between forb growth and the breeding success of quail (*1–3*), and it has long been presumed that the control is nutritional.

The breeding success of arid-land quail has been linked to storage in their livers of vitamin A obtained from green foods (*4, 5*). Whereas vitamin A is necessary for quail, there is no clear relation between the availability of this food supplement and the reproductive vigor in the birds (*6*). We surmise that there are other nutritional components of green food that regulate reproduction.

Phytoestrogens in subterranean clover inhibit breeding in domestic sheep (*7*). Thus, this group of compounds might reg-

Table 1. Effect of diet on egg production in three pairs of California quails.

Diet	Feeding period (month/day)	Onset of egg laying	Eggs per pair (No.)
Turkey starter*	10 March to 6 June	8 April	62
Low energy, low protein†	3 March to 30 June	8 May	28
Turkey starter plus subclover extract‡	3 March to 30 June	2 June	12

*Turkey starter contained 26 percent crude protein. †Low protein diet contained 15 percent crude protein. ‡Subclover extract contained biochanin A, genistein, and formononetin.