The Animal Cell Culture Collection[1, 2]

C. S. Stulberg, L. L. Coriell, A. J. Kniazeff, and J. E. Shannon[3, 4, 5]

Introduction

The widespread application of cell culture to problems in cancer research, cytologic and biochemical genetics, immunology, virology, toxicology, nutrition, and many other areas of biology and medicine has given rise to a cogent need for contaminant-free and well-characterized cell lines to serve as reference material for long term research. Recognition of this need led to the organization of the Cell Culture Collection Committee in 1960 under the leadership of the late J. T. Syverton and subsequent chairmanship by W. F. Scherer (1, 2, 3). Its purpose was to advise and aid a group of cooperating laboratories in the establishment and development of a national collection of reference animal cell cultures and to encourage research on the characterization and preservation of animal cell lines. The availability of such reference material to individual investigators and commercial organizations affords the following advantages:

1) The investigator can obtain "clean" standard materials at the same "passage" from time to time over the years for systematic experimentation.

2) He has a source of types of cells that may normally be difficult to acquire.

3) He is released from time-consuming and costly long-term serial subcultivation of cells.

4) He has insurance against the total loss of valuable cells through contamination, alteration, or accident.

The initial developmental problems in establishing the Collection were:

1) Development of freeze-preservation methods suitable for preparation and storage of reference seed stocks at ultra-low temperatures.

2) Establishment of viability criteria to judge the efficiency of recovery of cells from the frozen state.

3) Certification of reference cultures as being free from microbial contamination (particularly mycoplasmas), and development of methods of eliminating such contamination.

4) Identification of cells with regard to species of origin and intraspecies markers.

5) Characterization on the basis of karyology, morphology, growth characteris-

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[2] The Animal Cell Culture Collection was formed by the Cell Culture Collection Committee in 1960. This committee later became the Advisory Committee to the Animal Cell Culture Collection of the American Type Culture Collection (ATCC). Its present membership includes investigators from laboratories cooperating in the program and others as follows: C. S. Stulberg, (Chairman), L. L. Coriell, L. Hayflick, A. J. Kniazeff (for S. H. Madin), D. J. Merchant, H. R. Morgan, F. H. Ruddle, K. K. Sanford, R. E. Stevenson, and J. E. Shannon, *Secretary ex officio*.

[3] Dr. Stulberg is Senior Research Associate at the Child Research Center of Michigan, Detroit, and Professor of Microbiology, Wayne State University School of Medicine. Dr. Coriell is Director of the Institute for Medical Research (formerly the South Jersey Medical Research Foundation), Camden, New Jersey. Dr. Kniazeff is Head of the Cell Culture Laboratory, Naval Biological Laboratory (University of California), Oakland, Calif. Dr. Shannon is Curator of the Animal Cell Culture Collection at the American Type Culture Collection, Rockville, Maryland.

[4] Acknowledgments. The authors wish to thank members of the Committee as well as the following individuals for contributions and advice in the preparation of this article: F. H. Ruddle, W. D. Peterson, Jr., A. E. Greene, W. Nelson-Rees, M. Macy, F. Montes de Oca, W. W. Nichols, J. Charney, and W. F. Simpson.

[5] Reprints may be obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852.

tics, genetic and biochemical markers, virus susceptibility, tumorigenicity, and other special properties (2, 4, 5, 6, 7, 8).

Most of these developmental problems have been successfully solved (2). Cell cultures frozen in liquid nitrogen do not undergo appreciable loss of viability or other characteristics after cryogenic storage for 2.5 to 5 years (9). Over two thousand reference cultures have been successfully shipped from the Repository at the American Type Culture Collection to many laboratories throughout the United States and overseas.

As the program evolved, new information has been accumulated and new problems have become apparent. The Committee therefore revised the characterization and methodology used, and provided new guidelines for the selection of cell lines to be included.

Procedures and Criteria for the Certification of Reference Cell Cultures (1)

When an investigator wishes to submit a cell line for possible inclusion in the Animal Cell Culture Collection he may supply pertinent information about the cells on submission forms available from the ATCC. In each instance the history and genealogy of each cell line is supplied by the originator or his designate, and should include pertinent information such as: the species of origin, sex, race, age, tissue, initial isolation and culture techniques, subsequent data of significance, and of utmost importance, scientific significance of each cell line (including references to published literature). The Committee considers the merits and significance of each cell line proposed, and for those that are selected the investigator is asked to submit a representative seed culture to one of the participating laboratories for initial testing for microbial contaminants and verification of species, and preparation of reference seed stocks preserved in liquid nitrogen.

Cell lines selected for inclusion in the Collection are then fully characterized using the criteria listed below and when all criteria are satisfied, the cell line is certified by the Cell Culture Committee. Preliminary characterizations are done on aliquots of the original culture but, to insure accuracy, final characterizations listed in the catalog are carried out on representative ampules of the stocks from liquid nitrogen which are sent out to those requesting cell cultures from the Collection. The total number of subcultures of each cell line or strain and the total number of doublings or days in culture, when available, are designated. When the cells are fully characterized, the reference cells for distribution are prepared in sufficient quantity and suspended in a suitable antibiotic-free medium containing a cryoprotective agent such as glycerol or dimethylsulfoxide. The cells are then preserved by slow freezing at controlled rates and stored in liquid nitrogen. Characterization data include the following.

1. The comparative viabilities of the cell population just prior to freezing, and from representative frozen ampules, are determined by a dye-exclusion test or by growth response.

2. The medium requirements of the reference cultures are usually those employed in the isolation and/or continued propagation of the cells by the original investigator. The medium is antibiotic-free, and unless otherwise specified, contains fetal bovine serum.

3. Growth potential of cells is determined on known inocula of freshly thawed ampules. Cell yields in a given period of time are recorded.

4. When a cell line can be grown from single cells, direct plating efficiency from a thawed ampule of cells is determined.

5. The morphologic appearances of stained and/or living cells are recorded and photographed.

6. The diploid number of the species of origin (2n) is recorded, as well as the stem line number(s) of the cell line and, when applicable, deviations such as chromosomal polymorphism, aneuploidy, translocation or breaks. The chromosome frequency, distribution, and morphology

in at least 50 representative cells are determined, plus karyotype analysis of at least 10 cells within the modal number. An estimate of the frequency of polyploid cells in the population is established by comparison of total numbers of chromosomes in 100 to 200 metaphases under low power magnification. These observations are made on cells from the first or second passage following recovery from frozen preserved reference stock ampules.

7. Cell lines are tested and shown to be free of mycoplasmas, bacteria, fungi, protozoa, and cytopathic viruses. The presence or absence of virus-like particles by electron microscopy are recorded when appropriate.

8. The species of origin of the reference cell lines are verified by testing with specific antisera using one or more of the following tests or equivalent: mixed agglutination, fluorescent antibody reaction, indirect hemagglutination, cytotoxic-antibody dye exclusion and agar gel immunodiffusion. When distinct karyotypes clearly indicate species, these are used in conjunction with serological reactions.

9. A determination of susceptibility or insusceptibility to polioviruses is made as an indication of primate or nonprimate lines, and other viruses may be used for complete characterization.

10. Other specific characterization tests such as tumorigenicity, biochemical markers, drug susceptibility, isoenzyme analyses, electron microscopy, etc., are applied to reference cultures when indicated to verify their identities and characteristics.

11. Cell lines of limited life expectancies in culture are called finite cell lines and must meet all the criteria listed for permanent cell lines, but in addition must be shown to have a useful life expectancy after recovery from the frozen state.

12. Reference cells are usually prepared by the cooperating laboratories in batches of 100 to 200 ampules, each containing one to five million cells in 1 ml of medium. The cooperating laboratories are responsible for the accuracy of all characterizations and that the viability of the cells is adequate to permit recovery of a vigorous culture with the characteristics described.

The ATCC publishes and distributes lists and descriptions of cell lines (Table 1) which have been certified by the Committee (1). The cell lines receive accession numbers in the order in which they are accepted by the Committee, and the numbers are used for cataloging purposes. Each description contains the designation of the cell line, history, all characterization information, and pertinent references to permit ease of identification with previously published information. The recommendations of the Committee on Terminology of the Tissue Culture Association (10) have been followed as closely as possible. Variants or derivatives of cell lines are indicated by decimal annotation of the parent line, e.g.:

Cell repository number	
CCL 1	NCTC clone 929 (clone of strain L)
CCL 1.1	NCTC 2071 (CDM derivative of NCTC clone 929)
CCL 1.2	L-M (derivative of NCTC clone 929 L)

Investigators are urged to use the CCL number in publications relating to reference cells and also to designate the number of passages the cells are removed from the reference stock so that they may more readily compare results with other investigators.

Guidelines for Additional Study and Policy for the Selection of Cells

Since many new cell lines are constantly being developed, it is important that their current and potential value to the scientific community be critically evaluated. The policy guidelines for selection of cultures must reflect advances in biological research and the needs of the scientific community. Therefore it has been necessary for the Committee to make periodic reassessments of characterization criteria as well as the types of cells that should be added to the Collection.

TABLE 1

Registry of animal cell lines certified by the Cell Culture Collection Committee† as of April, 1968‡· ‖*

Name of Cell Line	Species or Other Classification	Cell Repository Number	Submitted by:
AtT-20 (pituitary tumor)	Mouse	CCL 89	G. H. Sato
AV_3 (amnion)	Human	CCL 21	F. C. Robbins
B14 FAF 28-G3 (peritoneal cells)	Hamster (Chinese)	CCL 14	T. C. Hsu§
B14-150 (peritoneal cells)	Hamster (Chinese)	CCL 14.1	T. C. Hsu
BHK-21 (C-13) (kidney)	Hamster (Syrian)	CCL 10	I. MacPherson
BS-C-1 (kidney)	Monkey (African Green)	CCL 26	H. E. Hopps
Bu (buffalo lung)	*Bison bison*	CCL 40	S. B. Dwight
CCRF S-180 II (sarcoma S-180)	Mouse (Hybrid albino)	CCL 8	G. E. Foley
Chang liver (liver)	Human	CCL 13	R. S. Chang
Citrullinemia (skin)	Human	CCL 76	W. G. Mellman
Dede (lung)	Hamster (Chinese)	CCL 39	T. C. Hsu
Dempsey (skin)	Human	CCL 28	T. C. Hsu
Detroit-6 (sternal marrow)	Human	CCL 3	C. S. Stulberg and L. Berman
Detroit-6, Clone 12 (sternal marrow)	Human	CCL 3.1	C. S. Stulberg and L. Berman
Detroit-98	Human	CCL 18	C. S. Stulberg and L. Berman
D98S (Detroit 98 with biochemical markers)	Human	CCL 18.1	W. Szybalski
D98/AG (8-azaguanine resistant)	Human	CCL 18.2	W. Szybalski
D98/AH-2 (8-azahypoxanthine resistant)	Human	CCL 18.3	W. Szybalski
D98/AH-R (8-azahypoxanthine sensitive)	Human	CCL 18.4	W. Szybalski
Detroit 532 (foreskin, Down's syndrome)	Human	CCL 54	C. S. Stulberg
Detroit 539 (skin, Down's syndrome, female)	Human	CCL 84	C. S. Stulberg
Detroit 529 (Skin; trisomy X and 21 groups, Down's syndrome)	Human	CCL 66	C. S. Stulberg
Detroit 525 (skin, XO plus centric fragment, Turner's syndrome)	Human	CCL 65	C. S. Stulberg
Detroit 510 (skin, galactosemia)	Human	CCL 72	C. S. Stulberg
Don (lung, diploid)	Hamster (Chinese)	CCL 16	T. C. Hsu
EBTr (embryonic bovine trachea)	*Bos taurus*	CCL 44	A. J. Kniazeff, W. A. Nelson-Rees and N. B. Darby, Jr.
E. Derm (equine dermis)	*Equus caballus*	CCL 57	A. J. Kniazeff, W. A. Nelson-Rees and N. B. Darby, Jr.
FHM (fat head minnow)	*Pimephales promelas*	CCL 42	R. G. Malsberger
FL (amnion)	Human	CCL 62	J. Fogh
FT (bullfrog tongue)	*Rana catesbeiana*	CCL 41	K. Wolf
GH_1 (pituitary tumor)	Rat	CCL 82	G. H. Sato
Girardi heart (heart)	Human	CCL 27	A. Girardi

TABLE 1—*Continued*

Name of Cell Line	Species or Other Classification	Cell Repository Number	Submitted by:
Grunt fin (GF) (salt-water blue-striped grunt)	*Haemulon sciurus*	CCL 58	M. M. Sigel
HaK (kidney)	Hamster (Syrian)	CCL 15	A. E. Moore§
HeLa (carcinoma, cervix)	Human	CCL 2	W. F. Scherer§
HeLa 229 (carcinoma, cervix)	Human	CCL 2.1	J. T. Syverton§
HEp-2 (carcinoma, larynx)	Human	CCL 23	A. E. Moore
I-10 (Leydig cell testicular tumor)	Mouse	CCL 83	G. H. Sato
Intestine 407 (embryonic intestine)	Human	CCL 6	W. Henle
J-111 (monocytic leukemia)	Human	CCL 24	E. E. Osgood
KB (carcinoma, oral)	Human	CCL 17	H. Eagle
L-132 (embryonic lung)	Human	CCL 5	E. V. Davis
LLC-MK_2 original (kidney)	Monkey (Rhesus)	CCL 7	R. N. Hull
LLC-MK_2 derivative (kidney)	Monkey (Rhesus)	CCL 7.1	R. N. Hull
LLC-WRC 256 (Walker rat carcinoma)	Rat (Harlan-Wistar)	CCL 38	R. N. Hull
L-M derivative of NCTC clone 929 (L)	Mouse (C3H/AN)	CCL 1.2	D. J. Merchant
MDBK (bovine kidney)	*Bos taurus*	CCL 22	S. H. Madin and N. B. Darby, Jr.
MDCK (canine kidney)	*Canis familiaris*	CCL 34	S. H. Madin and N. B. Darby, Jr.
MB III (de Bruyn-Gey) (Lymphosarcoma)	Mouse	CCL 32	G. O. Gey
MMT 060562 (mammary tumor)	Mouse (C57BL × Af)	CCL 51	J. Sykes
Mv 1 Lu (mink lung)	*Mustela vison*	CCL 64	A. J. Kniazeff, W. A. Nelson-Rees and N. B. Darby, Jr.
Minnesota-EE (esophageal epithelium)	Human	CCL 4	J. T. Syverton
NCTC clone 929 (clone of strain L)	Mouse (C3H/AN)	CCL 1	W. R. Earle§
NCTC clone 1469 derivative (liver)	Mouse (C3H/AN)	CCL 9.1	V. J. Evans
NCTC 2071 (CDM derivative of NCTC clone 929)	Mouse (C3H/AN)	CCL 1.1	V. J. Evans
NCTC clone 2472 (high tumor-producing line)	Mouse (C3H/HeN)	CCL 11	K. K. Sanford
NCTC clone 2555 (low tumor-producing line)	Mouse	CCL 12	K. K. Sanford
NCTC 2544 (skin epithelium)	Human	CCL 19	V. J. Evans§
NCTC 3075 (CDM derivative of NCTC 2544)	Human	CCL 19.1	V. J. Evans§
NCTC clone 3526 (CDM derivative of LLC MK_2)	Monkey (Rhesus)	CCL 7.2	V. J. Evans§
NCTC 3749 (CDM derivative of lymphoid neoplasm P388 D_1)	Mouse (DBA/2)	CCL 46.1	V. J. Evans§
NCTC 4206 (CDM derivative of B14 FAF 28 G)	Hamster (Chinese)	CCL 14.2	V. J. Evans§
NCTC 3959 (Cloudman S91 A amelanotic melanoma)	Mouse (C × DBA)	CCL 52	K. K. Sanford
NCTC 3960 (Cloudman S91 melanotic melanoma)	Mouse (C × DBA)	CCL 53	K. K. Sanford
PK (15) (porcine kidney)	*Sus scrofa*	CCL 33	Cutter Laboratories
Pt K1 (kidney) (female)	Marsupial, *Potorous*	CCL 35	K. H. Walen

TABLE 1—*Continued*

Name of Cell Line	Species or Other Classification	Cell Repository Number	Submitted by:
Pt K2 (kidney) (male)	Marsupial, *Potorous*	CCL 56	K. H. Walen
RPMI 1846 (melanotic melanoma)	Hamster (Syrian)	CCL 49	G. E. Moore
RPMI 2650 (quasi-diploid tumor)	Human	CCL 30	G. E. Moore
RR 1022 (Schmidt-Ruppin sarcoma)	Rat (Norway)	CCL 47	W. W. Nichols
RT-2 rainbow trout (gonadal tissue)	*Salmo gairdneri*	CCL 55	K. Wolf
SIRC (Statens Seruminstitut rabbit cornea)	*Oryctolagus cuniculus*	CCL 60	J. Leerhy§
TH-1, Sub B1 (heart, terrapene turtle)	*Terrapene carolina*	CCL 50	H. F. Clark
Tu Wi (Wilms's tumor)	Human	CCL 31	J. Leighton§
WI-38 (lung, diploid)	Human	CCL 75	L. Hayflick
WISH (amnion)	Human	CCL 25	L. Hayflick
Wong-Kilbourne derivative (D) of Chang conjunctiva (clone 1-5c-4)	Human	CCL 20.2	E. D. Kilbourne§
Y-1 (adrenal tumor)	Mouse	CCL 79	G. H. Sato

* Each of the reference cell lines listed are deposited in the repository at the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20852. Catalogues and supplements containing detailed individual characteristics of the lines, including instructions for their procurement, may be obtained from the ATCC.

† This committee was officially designated "The Advisory Committee of the Animal Cell Culture Collection to the American Type Culture Collection".

‡ As of this date over 2000 reference cultures have been requested by the scientific community.

§ In these instances the individuals who isolated the original lines are indicated in the catalogue (1).

‖ References to each of these cell lines and related work will be found in the catalogue (1).

ADDENDUM

TABLE I (continued)

*Registry of animal cell lines certified by the Cell Culture Collection Committee between May 1, 1968 and June 1, 1969**†‡§‖

Name of Cell Line	Species or Other Classification	Cell Repository Number	Submitted by:
A6 (kidney; South African clawed toad)	*Xenopus laevis*	CCL 102	K. A. Rafferty, Jr.
BF-2 (Bluegill Fry)	*Lepomis machrochirus*	CCL 91	K. Wolf
C-6 (glial tumor)	Rat	CCL 107	G. Sato
Car (adult goldfish)	*Carassius auratus*	CCL 71	L. Moewus-Kobb and M. M. Sigel
CCRF-CEM (Peripheral blood; acute lymphoblastic leukemia)	Human	CCL 119	G. E. Foley
CCRF-SB (peripheral blood; acute lymphoblastic leukemia)	Human	CCL 120	G. E. Foley
CCRF-HSB-2 (Human tumors in hamsters; source peripheral blood, acute lymphoblastic leukemia)	Human	CCL 120.1	G. E. Foley
Ch 1 Es (NBL-8) (goat esophagus)	*Capra hircus*	CCL 73	A. J. Kniazeff, *et al.*
Clone M-3 (Cloudman S91 melanoma)	Mouse (C × DBA)	CCL 53.1	G. Sato
Cri du Chat (skin)	Human	CCL 90	W. R. Breg
CV-1 (kidney)	Monkey (African Green)	CCL 70	F. C. Jensen
Detroit 548 (partial D trisomy translocation, skin)	Human	CCL 116	C. S. Stulberg
Detroit 550 (foreskin)	Human	CCL 109	C. S. Stulberg
Detroit 551 (embryonic skin)	Human	CCL 110	C. S. Stulberg
Detroit 573 (B/D translocation, skin)	Human	CCL 117	C. S. Stulberg
EB-3 (Burkitt lymphoma)	Human	CCL 85	W. Henle (Epstein)
IgH-2 (iguana heart)	*Iguana iguana*	CCL 108	H. F. Clark
JDU (galactosemic, skin)	Human	CCL 118	R. S. Krooth
Jijoye (Burkitt lymphoma) clone P-2003	Human	CCL 87	W. Henle
LLC-RK_1 (kidney; rabbit)	*Oryctolagus cuniculus*	CCL 106	R. N. Hull
P1 1 Ut (NBL-9) (raccoon uterus)	*Procyon lotor*	CCL 74	A. J. Kniazeff, *et al.*
R2C (Leydig cell testicular tumor)	Rat (Wistar-Furth)	CCL 97	G. Sato
RAJI (Burkitt lymphoma)	Human	CCL 86	W. Henle (Epstein)
Sf 1 Ep (NBL-11) (epidermis, cottontail rabbit)	*Sylvilagus floridanus*	CCL 68	A. J. Kniazeff, *et al.*
Tb 1 Lu (NBL-12) (bat lung)	*Tadarida brasiliensis*	CCL 88	A. J. Kniazeff, *et al.*
Vero (kidney)	Monkey (African Green)	CCL 81	W. D. Hann and J. S. Rhim
3T3 (embryo fibroblasts)	Mouse (Swiss albino)	CCL 92	H. Green

* Eight of the cell lines listed in this table are described in the 3rd Supplement of the Registry of Animal Cell Lines certified by the Cell Culture Collection Committee, 1968 (see Ref. 1, Table 1). The remainder of the cell lines listed above have been certified by the Committee, and are available from the cell repository of the American Type Culture Collection. Complete descriptions of these will appear in the 4th Supplement of the Registry which is currently in preparation.

† As of this date over 3000 reference cultures have been requested by the scientific community.

‡ The Animal Cell Culture Collection of the ATCC has been designated as the World Health Organization International Reference Center for cell cultures for the WHO Virus Reference Center.

§ Dr. A. J. Kniazeff's present address is: Institute for Comparative Biology, P.O. Box 551, San Diego, California.

‖ Since the original manuscript was prepared, a number of new advances in cell culture have appeared in the literature. However, many of these are covered in Differentiation and Defense Mechanisms in Lower Organisms, 1967, In Vitro, *3:* 3–199, and in Hemic Cells, 1968, In Vitro, *4:* 3–182.

Identification of Cell Lines

One of the most obvious problems requiring solution in the Animal Cell Culture Collection program has been that of identification of cell lines. Many investigators have felt that the most satisfactory method for identifying individual cultures would be through the use of genetic markers, if such were available. It has been possible to identify cells with regard to species of origin by immunological (11, 12, 13, 14, 15, 16, 17, 18) or cytogenetic (19, 20, 21) means, but intraspecies identification of many species has rarely been possible (22, 23, 24) except for euploid cells containing chromosome markers and biochemical deficiencies (21, 25, 26). However, the development of isoenzyme (isozyme) techniques (27, 28, 29, 30) and the recognition of genetically determined enzyme polymorphisms has opened the possibility of utilizing these as stable genetic markers. Isozymes are variant forms of enzymes which differ in their electrophoretic mobilities, but which remain unaltered in their activities and substrate specificities. Many of these variant forms of enzymes are genetically determined, and can occur at high frequencies in animal populations as polymorphisms. Recent studies (28, 31) have shown that the incidence of isozyme polymorphisms in several different species is quite high, and the number ascertainable by electrophoresis for any particular species is potentially large. Since such isozyme polymorphisms are frequently well expressed in cultured cell populations, it suggests that they might be employed for marking and identifying cell populations which have arisen from different individuals within the species.

Gartler (27) found that most human heteroploid cell lines contain the same variant form of glucose-6-phosphate dehydrogenase. He suggested that many of the heteroploid human cell lines developed by investigators may be derivatives of HeLa cells through contamination. This suggestion was based on the occurrence of G6PD type A+ and PGM type 1 isoenzymes in 100% of the permanent cell strains he examined. HeLa cells were of Negro origin and the type "A" variant of G6PD is found in less than 30% of the Negro population and never in Caucasians.

The important consequences of these findings made it desirable to verify and extend similar studies of the human cell populations in the Collection. In a cooperative study three of the participating laboratories independently studied cells in the Collection as well as other cell populations. The following electrophoretic methods were used: starch gel, acrylamide gel and a more recent procedure (32) using acetate or sucrose gel film. The results of this study are shown in Table 2.* Of the permanent cell lines examined, 24 exhibited the fast moving "A" electrophoretic variant of G6PD. Only 9 lines exhibited the slower moving "B" variant and 8 of the 9 lines were provisionally regarded as having a finite life expectancy. In summary, these results confirm the experimental results of Gartler (27) and thus far are consistent with his interpretation. This has an important bearing on the problem of whether neoplastic transformations can occur from normal human cells *in vitro*. To obtain additional data on this problem, cell lines have been established (32) in which diploid and near-diploid lines contain the "A" type isoenzymes of G6PD, the "B" type isoenzyme of G6PD, a mosaic containing both the "A" and "B" isoenzymes, and a heteroploid epithelial-like cell line containing the "B" type of isoenzyme. These cell lines appear to remain stable for their G6PD variants but are being observed over long term culture to provide experimental evidence to clarify the stability of these isoenzymes *in vitro*. Although many more isoenzyme polymorphisms need to be studied, Gartler has performed a great service by focusing the attention of cell culturists on the importance of the positive identification of cells within a species.

* See also Fig. 1, page 11.

TABLE 2

*Isoenzyme type of glucose-6-phosphate dehydrogenase found in human cell lines by three electrophoretic methods**

Name of Cell Line	CCL Number	Description of Cell Line	Racial Origin of Donor	Electrophoretic Method: 1,† 2,‡ 3§	G6PD Type	
					A	B
AV_3	21	Heteroploid, Ep-L, permanent	Negro	1, 3	+	
Chang liver	13	Heteroploid, Ep-L, permanent	?	1, 2	+	
Citrullinemia	76	Diploid, Fb-L, finite	?	1, 2		+
Dempsey	28	Diploid, Fb-L, finite	Caucasian	1		+
Detroit-6	3	Heteroploid, Ep-L, permanent	Caucasian	1, 2, 3	+	
Detroit-6, clone 12	3.1	Heteroploid, Ep-L, permanent	Caucasian	1, 2	+	
Detroit-98	18	Heteroploid, Ep-L, permanent	Caucasian	1, 3	+	
D98s	18.1	Heteroploid, Ep-L, permanent	Caucasian	1, 3	+	
D98/AG	18.2	Heteroploid, Ep-L, permanent	Caucasian	1, 3	+	
D98/AH-2	18.3	Heteroploid, Ep-L, permanent	Caucasian	1, 3	+	
D98/AH-R	18.4	Heteroploid, Ep-L, permanent	Caucasian	1, 3	+	
Detroit 532	54	Near-diploid, Fb-L, finite	Caucasian	1, 3		+
Detroit 539	84	Near-diploid, Fb-L, finite	Caucasian	3		+
Detroit 529	66	Near-diploid, Fb-L, finite	Caucasian	1, 3		+
Detroit 525	65	Near-diploid, Fb-L, finite	Caucasian	1, 3		+
Detroit 510	72	Near-diploid, Fb-L, finite	Caucasian	1, 3		+
FL	62	Heteroploid, Ep-L, permanent	?	1, 3	+	
Girardi heart	27	Heteroploid, Ep-L, permanent	?	1	+	
HeLa	2	Heteroploid, Ep-L, permanent	Negro	1, 2, 3	+	
HeLa 229	2.1	Heteroploid, Ep-L, permanent	Negro	1, 2	+	
HEp-2	23	Heteroploid, Ep-L, permanent	Caucasian	1, 2, 3	+	
Intestine	6	Heteroploid, Ep-L, permanent	Caucasian	1, 2	+	
J-111	24	Heteroploid, Ep-L, permanent	?	1, 2	+	
KB	17	Heteroploid, Ep-L, permanent	Caucasian	1, 2, 3	+	
L-132	5	Heteroploid, Ep-L, permanent	?	1, 2	+	
Minnesota-EE	4	Heteroploid, Ep-L, permanent	?	1, 2, 3	+	
NCTC 2544	19	Heteroploid, Ep-L, permanent	Caucasian	1, 3	+	
NCTC 3075	19.1	Heteroploid, Ep-L, permanent	Caucasian	1	+	
RPMI 2650	30	Near-diploid, Ep-L, permanent	Caucasian	1, 2		+

TABLE 2—*Continued*

Name of Cell Line	CCL Number	Description of Cell Line	Racial Origin of Donor	Electrophoretic Method: 1,† 2,‡ 3§	G6PD Type	
					A	B
Tu Wi	31	Heteroploid, Ep-L, permanent	?	1	+	
WI-38	75	Diploid, Fb-L, finite	Caucasian	1, 2, 3		+
WISH	25	Heteroploid, Ep-L, permanent	Caucasian	1, 2, 3	+	
Wong-Kilbourne	20.2	Heteroploid, Ep-L, permanent	?	1	+	

* Cooperative studies of three participating laboratories of the Cell Culture Collection.
† 1: Starch gel procedure carried out at the ATCC.
‡ 2: Acrylamide gel procedure carried out at the Institute for Medical Research.
§ 3: Sucrose-gel method (32) carried out at the Child Research Center of Michigan.

Microbial Contamination

The experience of the cooperating laboratories of the Cell Culture Collection has shown that a great many of the cell cultures submitted are contaminated by bacteria or mycoplasmas (33, 34). Most of these contaminations were not suspected by the submitting investigator but were readily revealed by removing the antibiotics from the culture media. It seems fair to conclude that contamination of cell cultures is still a major problem and a serious one. The cooperating laboratories, with the encouragement of the Committee, are developing improved procedures and equipment for the prevention, control and elimination of microbial contamination of cell lines. It seems quite clear that many of the contaminants probably occur via the airborne route. Recent studies (35, 36) have shown that modern air filters and laminar flow hoods aid measurably in providing a much more suitable aseptic environment for cell culture work than has been conventionally employed. The high efficiency particulate air (HEPA) filters remove 99.97% of dust particles larger than 0.3 microns in diameter. Open culture flasks in this environment remain sterile for hours. Filtered air in a hood or transfer room sampled at the rate of 1 cubic foot per minute is essentially sterile when tissue cultures are being transferred. This is in contrast to the many bacteria removed from the air of a conventional transfer room.

Selection of Cell Lines on the Basis of Special Characteristics and Uses

At first only permanent cell lines were added to the Collection either because of demonstrated usefulness in investigative research or because of historical value as prototypes. It seems clear, however, that most cultured cells fall rather sharply into one of three categories (1, 25, 37):

1) Those that show considerable variation, are heteroploid, grow indefinitely as permanent cell lines, and may be epithelial-like (human) or fibroblast-like (other species).

2) Those that show little variation, retain the karyotype of the donor, have a finite life span of about 20 to 50 passages, are non-malignant, and are usually fibroblastic.

3) Those often isolated from peripheral blood, diploid in karyotype, are seemingly permanent, fail to attach to glass or plastic surfaces, and are "lymphoid" in morphology. The latter have been isolated from humans in cases of acute leukemia in children and adults (38, 39, 40, 41), from normal individuals (42), from Burkitt's lymphoma (43) and from infectious mononucleosis (44).

Permanent or Established Cell Lines

The permanent or established cell lines already in the Collection, whether of hu-

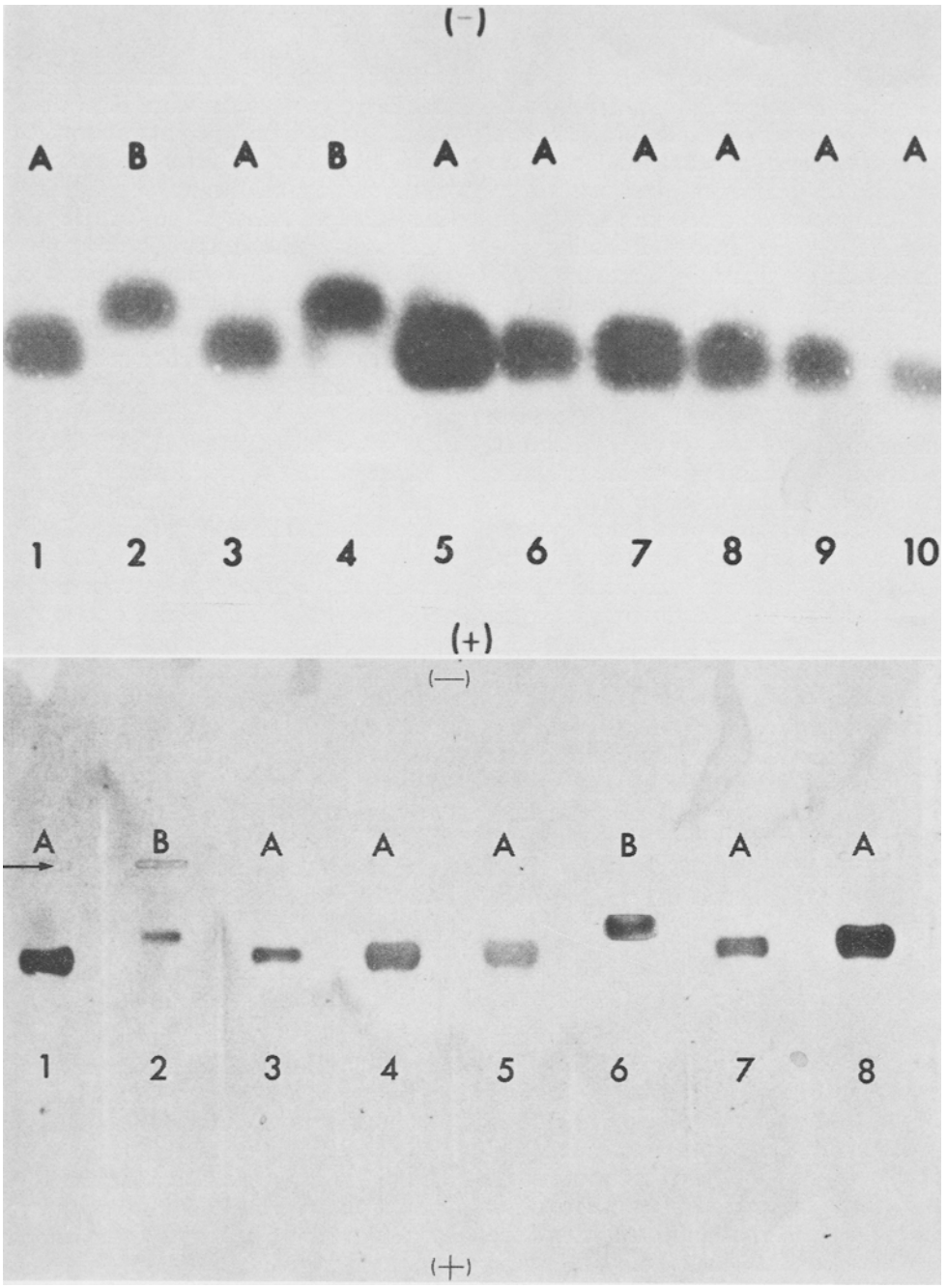

FIG. 1. *Top:* G6PD Isoenzyme patterns of CCL lines by starch-gel electrophoresis. Origin is at the cathode (−). "A" indicates the fast-moving isoenzyme; "B" indicates the slower moving form. 1) HeLa; 2) Det. 532; 3) Chang conjunctiva (Wong-Kilbourne derivative); 4) Dempsey; 5) AV_3; 6) FL; 7) NCTC 3075; 8) NCTC 2544; 9) WISH; 10) WISH (CDM derivative). *Bottom:* G6PD Isoenzyme patterns of CCL lines by sucrose-gel film electrophoresis (32). The arrow indicates the origins. "A" indicates the fast-moving isoenzyme; "B" indicates the slower moving form. 1) HeLa; 2) Det. 532; 3) Det. 6; 4) AV_3; 5) KB; 6) Det. 525; 7) FL; 8) Det. 98.

man origin or from other species, have been useful reference material for a multitude of purposes in biological research. Besides the human cells, the Collection now includes permanent lines established from common laboratory animals, domestic animals, wild species including primates, rodents, marsupials, martens, fish, amphibians, and reptiles (Table 1). Selected permanent cell lines from any species will continue to be considered by the Committee for possible addition to the Collection provided the cells exhibit unique characteristics or are of special usefulness in other ways to the scientific community. Examples of the special uses considered are: tumor production, possession of an oncogenic virus or antigens, chromosome markers, biochemical defects, production of hormones, melanin, mucin, origin of blood group substances, drug sensitivities and unique viral susceptibilities (1, 45, 60, 62). Selected cells adapted to growth in protein-free chemically defined media that are of usefulness in nutritional and biochemical studies and in the evaluation of the effects of hormones, drugs, toxic agents, viruses and chemical carcinogens (46, 47, 48, 49, 50, 51) have been included and others will be considered.

Diploid or Near-Diploid Cells with Finite Life Expectancy

The significance of the diploid finite cell lines originating from human fetal tissues has been pointed out by Hayflick (26, 37). He emphasized their importance as substrates for virus propagation and for the preparation of virus vaccines in comparison to permanent epithelioid and heteroploid cell lines. In recent years finite cell lines have had far-reaching effects in oncology (particularly viral) and in virology, and are of great importance in the field of mammalian cell genetics (25). Thus a new phase of the Animal Cell Culture Collection was initiated: the accessioning of finite strains developed by interested investigators *and* by the participating laboratories to meet the needs in various fields. Such strains include human and animal cells with both normal and abnormal karyotypes, cells with biochemical genetic markers and polymorphisms, cell strains adaptable to studies of virus transformations, and cell strains with significant virus susceptibilities. Eagle (52, 53) has demonstrated the metabolic controls in such cells (as well as in permanent cell lines) and has shown how they might provide an approach to a better understanding of regulation and function in the whole animal. Examples of the uses of finite cell lines have also been elaborated upon by many other investigators (37, 25, 52).

In the interest of providing the scientific community with such valuable research material the Animal Cell Culture Collection has developed protocols for the preparation of reference seed stocks at low passage levels. The patterns for the procedures that have been adopted were established by Hayflick and co-workers (37), by the cooperating laboratories of the Collection, and by numerous other investigators. A series of cultures, derived from one or more tissues, are carried through the third to the fifth passage depending on growth rate or other factors. At such levels, a culture possessing the most desirable growth characteristics and requisite cytological, biochemical, or other characteristics is selected for further development. The selected culture is expanded to the lowest possible passage level from which at least 50 or more ampules of frozen characterized reference cells can be prepared. The cells are expanded further in order to determine their life span. Meanwhile, samples are preserved at designated passage levels for characterizations to be repeated. By following such procedures it is possible to determine the highest passage level at which the culture would be considered useful for experimental purposes. Any spontaneous or experimental changes of any nature occurring during successive passages can be verified by reference to the original seed stocks in the repository. If some of the short-term cell lines develop into permanent cell strains with special useful characteristics, these strains might also be

added to the Collection at a later date. In this event, the reference seed stock from earlier passage levels would be available for comparative studies. The above procedures have been followed by the participating laboratories of the Animal Cell Culture Collection and other laboratories to establish a series of cell lines with a finite life expectancy from a variety of human and other animal species (Table 1).

Human and/or Animal Lymphoid Cells, or Cells Derived from Peripheral Blood

A recent advance in the cultivation of human cells has been the development of seemingly permanent cell lines from hematopoietic and/or lymphatic origins. These have been reported from normal peripheral blood (36), from lymphatic leukemia (38, 41), from myelogenous leukemia (39, 40), and from Burkitt's lymphoma (43). Some have already been characterized and placed in the Collection while others are in process. Many have the unique feature of being started from buffy layers of blood in suspension cultures. They are also of a diploid or occasionally near-diploid nature. Those associated with human sources as well as many derived from murine leukemias contain virus or virus-like particles as determined by electron microscopy or by isolation. Others contain antigens recognizable by a variety of means (54, 55, 56). In the last few years some lines have been reported to produce certain types of immunoglobulins and thus may provide valuable reference material for immunologic and related transplantation studies (57, 58). An important recent finding indicates a relationship between some of the particles of human cells and infectious mononucleosis (44). The cell source, morphology, manner of growth, virus particles, and karyotype make such lines important candidates for studies of many types of biologic phenomena and thus will be considered and characterized for the Collection. The Collection will expand this activity as the significance of such lines becomes better known.

Biochemical and Genetic Variant Cell Strains

The utilization of cell populations in comparative biochemical studies and genetic analysis has increased greatly in recent years. The Collection has already included (Table 1) a number of cell lines suitable for studies of this nature. The addition of biochemical and genetic variant cells was stimulated largely as the result of a comprehensive field survey made for the Committee by F. H. Ruddle. The inclusion of such cells, removed only 10 to 20 serial subcultures from the human or animal of origin, affords to investigators a variety of important and rare reference material that heretofore was not generally available for intensive research. Cells have been accessioned that were derived from individuals afflicted with diseases characterized by abnormalities of the human karyotype, such as Down's syndrome, Turner's syndrome, Klinefelter's syndrome and others (Table 1). The Collection also includes cells derived from individuals with genetically associated biochemical deficiencies such as galactosemia and citrullinemia (Table 1). These biochemical deficiencies are demonstrable in the cultured cells, thus providing a means of studying the defect in the living cells under controlled conditions *in vitro*. Other variant cells considered to be important as reference material will be added as they become available.

Virus Transformed Cells

Because of their wide interest and potential importance (59) in understanding virus-cell interaction in cancer, selected cell lines which have undergone transformation (possibly malignant) as a result of infection with tumor viruses, will be deposited in the Cell Registry and stored under suitable conditions to prevent possible tumor virus contamination of cell lines now in the Registry. A few lines of this type are already in the Collection (Table 1). In considering additional lines preference will be given to paired cell lines, *i.e.* control non-transformed cells and transformed cells. Selection of such

cell lines will be made on the basis of recommendations solicited from specialists in the field.

Summary

The Animal Cell Culture Collection established by the Advisory Committee and cooperating laboratories at the American Type Culture Collection has been described. The description includes procedures and criteria for the acceptance and certification of cells, guidelines for future studies, and policies for the selection of cells. The aim of this Collection is to fulfill the needs of individual investigators for characterized reference cells in broad areas of biological and medical research. The aid of interested investigators in anticipating and meeting these needs is earnestly solicited.

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