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Biological Risks from work with Primary Human and Established Cell Lines

1. Introduction

One of the key aspects of controlling biological risk is identifying the potential for harm presented by living material of biological origin. For infectious microorganisms and some multicellular parasites there is a well-established framework of classifying the risk into different risk groups. WHO produces guidance on the definition of risk groups, and various national jurisdictions produce their own definitive lists of organisms assigned to different risk groups reflecting local conditions.

However very frequently in research it is necessary to work with cell lines, which while alive are not of themselves infectious. The vast majority of cell lines are of low biological risk, but a small number do represent a risk because the way they have been derived means that they are a potential source of various viruses and other pathogens, which are infectious. This document provides guidance and some resources to help identify the risk presented by cell lines.

Guidance

Cells and tissues recently present in a human body and established cell lines represent different potential risks.

Human origin cells, even those derived from very aggressive tumours are not a biological safety risk as such. This is because they will be recognised as foreign by the immune system of other humans they may come into contact with and be rejected. Most tumour cells arise from mutations or chromosomal rearrangements which are non-transmissible. Thus most established cell lines will represent low biological risk. This highlights that it is very inadvisable to ever culture your own cells or cells of any other colleagues who are using the same cell culture suite. Under these circumstances cells would not be recognised as foreign, which is the main assumption for designating these types of cell as low risk.

A small number of important cell lines are the product of viral transformation and can produce active virus; they are higher risk. Some cell lines useful for hepatocellular carcinoma (HCC) research fall into this category. While harmless to humans some cell lines harbour viruses that are infectious to animals, particularly to laboratory mice. Because such cell lines may be introduced into mice for experimental purposes it is important to recognise this risk.

When material is directly isolated from patients or other donors a different potential risk is present. Such material may carry pathogens resulting from infections the donor may or may not be aware of. In particular because of the high prevalence of Hepatitis B in southern China (5%), which is transmitted via blood, out of caution it is best to assume that all human origin material is a potential hepatitis B and other infectious agent risk. This means that such primary material needs to be considered a hazard group 2 agent and handled under at least Biosafety Level 2 conditions.

Attempts to conduct multiple serial passages of primary cultures are unlikely to produce conditions susceptible to virus propagation for most cell types. Most intentional viral culture requires knowledge of a specific cell type that is infectible. Thus in general serial passage to yield a particular cell type or organoid for further work is likely to dilute any viruses present rather than sustain them. Thus after sufficient in vitro passages, usually three, it can be assumed that a primary culture no longer presents an infection risk and can be handled under BSL-1 conditions. However, there are important exceptions to the concept that repeated culture of primary cells will render them pathogen free. Because of the high incidence of hepatitis B infection it is advisable to regard hepatocyte cultures as high risk after multiple passages unless clear evidence of their viral status is available. Similarly maintenance of lymphocytes is potentially able to maintain lentiviruses such as HIV, hence primary lymphocyte cultures should be regarded as intrinsically hazardous.

Safety Office Lists

In order to help researchers Safety Office maintains two lists of cell lines, those which are low risk (the vast majority) and a much shorter list of cell lines that do represent a risk. The way early cell lines were obtained means that some heavily used in research have considerable potential risk associated with their use. In the past it was a challenge in some cases to find any cell lines with properties representative of particular cell types. Because of this some early cell lines were produced by techniques such as mechanically shearing viral DNA, introducing it to diploid cells and the selecting for clones that could replicate indefinitely in vitro. As this took place in the days when DNA sequencing was a relatively novel technique, and genome sequencing was yet to take place, frequently such cell lines were characterized to the extent that some viral-derived sequences were shown to be present, with no further characterization since. Where information is limited it is necessary out of caution to assume that the full sequences of the viruses used to produce the cell line *may* be present. In other cases cell lines were produced by culturing tumour cells derived from the tissue of interest, in situations where tumours are frequently caused by a transforming virus. Once again the sequences of a virus *may* be present. In such cases, unless there is clear evidence to the contrary, it is necessary to assume that such cells can potentially produce infectious virus, and therefore to culture the cells is to culture the virus. Some biological supply organisations will not guarantee that the cell lines they supply are free from contamination with infectious agents. It is necessary to assume that risk is present because

of the source of the cells, not based on the likely risks represented by that cell line.

However from time to time particular cell lines become better characterized, for example when whole genome sequences are obtained, or an experiment is performed in such a way that the presence of infectious virus particles would be revealed. At that point it is possible to demonstrate that while viral sequences are present, either the whole viral genome is not or it is damaged or in the case of some virus types integrated in such a way that it cannot produce an infectious particle. When such evidence comes to our attention we will revise our lists. On a small number of occasions cell lines have been moved from the 'high risk' to the 'low risk' category. If users are aware of published literature or data relating to cell lines that are currently considered high risk please share this with the biosafety team of safety office.

If a cell line that does not appear on these lists is required please contact safety office for assistance in performing a risk assessment to determine whether it is high risk or low risk.

Risk Controls for Cell Line Work

The advice in this document only considers the risk controls required for the cell lines themselves. If the experimental strategy involves modification of the cell line then the nature of the modification may determine the risk level of the cells. A GM risk assessment will be needed in this case. One example scenario when this would be necessary is when a cell line is modified to produce infectious virus by introduction of a molecular clone encoding complete viral sequences. In this case the risk from the cell line would be determined by the virus it is engineered to produce. Another example is when a cell line is used to produce a cancer model by transfection of plasmids encoding known oncogenes. As long as the plasmids are non mobilizable then the cell line itself does not represent an enhanced risk after it is modified. However there is a level of risk associated with the process by which the cell line is exposed to oncogenic plasmids, which needs to be reflected in a GM risk assessment.

For low risk cell lines standard lab precautions and good sterile cell culture technique are all that are required. Low risk cell lines can be flow sorted in the open lab. For work with high risk cell lines BSL-2 lab arrangements are required. Briefly these include SOP's for all tasks in the laboratory including cell culture work, an SOP for disinfection and spillage, a spillage kit available in the lab matched to the maximum size of cultures and an in-date disinfectant, secondary containment for cell cultures while in the incubator, labelling of storage areas with the biohazard symbol and indication of risk eg. human material, hepatitis B risk etc., centrifuges with sealable rotors and buckets that can be loaded and unloaded within an MSC, access to the lab restricted to those trained on the SOP and an autoclave validated for waste disposal available, preferably on the same floor, for emergency decontamination after an accident. Cell sorting of high risk cell lines requires a cell sorter housed within a purpose-designed microbiological safety cabinet. This is because of the intrinsic risk of blockage and then sudden release at the culture aspiration point, leading to a large volume of infectious aerosol production.

Higer Risk Cell Lines December 2024

Risk Group 2 cell lines ECACC	Risk Group 2 cell lines (known agents)	Risk Group 2 cell lines (potential agents)
A2780ADR	22Rv1 (XMRV - human xenotropic retrovirus)	C666-1 (EBV)
CMT-167	AGS gastric cancer (parainfluenza type 5)	CLC7 (HBV)
LLC	C17 (EBV)	H2M (HBV)
OAW28	HEK293F(adenovirus)	H2P (HBV)
	HEK293FT(adenovirus)	H4M (HBV)
	HEK293T(adenovirus)	MHC97H (HBV)
	HEK293TN(adenovirus)	MHCC-97 (HBV)
		MHCCLM3 (HBV)
	KYSE-70 (XMRV)	NPC43 (EBV)
	MEC2(EBV)	PDX#1 (HBV)
	MIHA (HBV)	PDX#5 (HBV)
	PLC8024 (HBV)	SNU423 (HBV)
	RAW 264.7(Ab-MLV)	SNU449 (HBV)
	RM-1 mouse xenotropic retrovirus transformed	SUNE1 (EBV)
	SiHa (HPV)	
	Vcap mouse xenotropic retrovirus Bxv-1	

Risk Group 1 cell lines December 2024

4T1
81-T
A2058
A253
A375
A549
A-673
AB1
AH130
AK-47
ALL-SIL
AsPC1
B16-F10
B16-OVA
BA/F3
BEL7402(HeLa)
BNL CL.2
BS-C-1
BT-474
BT-549
BxPC3
C26
C2C12
C4-2
C666
Caco-2
Calu-3
CCRF-CEM
CEM
CHO
COLO 205
CT26
D1642
DF-1
E.G7-OVA
E0771
E5054
EL4
EL4
Es2
ES2
ESCC-luc
EW8
FaDu
GL261
H1650
H1975
H1-hESC
H2052
H2228
H2452
H28
H292
H3122
HaK
HCC1806
HCC827
HCT 116
HEK293
HEK293E
HeLa
Hep3B (HBV)
Hepa1.6
hepg2
HepG2
HEY A8
hFOB 1.19
HGS1
HGS4
HKSC-1
HL-60
HLE
Hs 578T
HT-1376
HT-29
Huh1(HBV)
huh7
Huh7
ID8
IMR-32/imr-32
JM
Jurkat
K562
Kasumi-1
KATO-III
KE37
KG-1
KKU213
KPC
KPC
Kuramochi
KY821
KYSE-140
KYSE-150
KYSE-180
KYSE-270
KYSE-30
KYSE-30
KYSE-410
KYSE-450
KYSE-510
KYSE-520
LCC2
LD-1
LLC (if from ATCC)
LM1
LNCaP
LOUCY
MB49
MC-38
MCF10A
MCF-7
MDA-MB-231
MDA-MB-453
MDA-MB-468
MDCK
mEC25
mEC35
MHCC97L
MHCCLM3
MiaPaCa-2
Miapapc-2
MKN-45
ML-1
ML-2
MOC1
MOLM-13
MOLT16
MOLT4
MOSE
MOVCAR
MSTO-211H
Mv-11
MV4-11
N1E115
NALM-6
NB-1
NB4
NCI-H460
NCI-N87
Neuro2a
NIH-H460
NOMO-1
NP69(but large T antigen)
OCL-AML3
OVCA420
OVCA433
OVCAR3
OVSAHO
PALL-1
Panc02
PANC1
PC-3
PEER
PEO1
PLC/PRF/5
PME1
RBE
REN
Renca
RIL175
RPE1
S2-VP-10
Saos-2
SCC-9
SH-SY5Y
SKBR3
sk-HEP1
SK-Mel-28
SK-N-BE(2)
SK-N-LP
SKOV3
SKOV-3
SK-UT-1
SLMT-1
SMMC7221 (HeLa)
SNU-16
SNU-I
SSP25
SUM149
SUM190
SW1353
SW1990
SW480
SW-684
SY5Y
T47D
TF1
THLE2(SV40)
THP-1
TIB-223
U-2932 DLBCL
U87MG
U-937
UACC903
UM-SCC-1
Vero
VH64
WA01
WERI-Rb-1
WM266-4
Y79
YTN16