Subculturing for adherent cell lines

No	Description/Details of Steps in Activity	Hazards	Possible Accident / Ill Health & Persons-at-Risk	Existing Risk Control (Mitigation)	Severity	Likelihood (Probability)	Risk Level	Additional Risk Control
1	Remove and discard old media. Avoid disturbing the adhered cells.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens,	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury	All personel handling cell lines are to have Hep B vaccination, and passage numbers are not to be maintained too high. Internal training is compulsory. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand.	1	1	2	
2	Wash flask/dish with 10 ml of DMEM (-), then remove. Avoid washing off cells.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens,	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury	All personel handling cell lines are to have Hep B vaccination, and passage numbers are not to be maintained too high. Internal training is compulsory. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand.	1	1	2	
3	Trypsinize the cells by adding 2 ml of DMEM (-) and 1ml of trypsin. Swirl the flask/dish, then incubate for 4 – 7 minutes.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens,	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury	All personel handling cell lines are to have Hep B vaccination, and passage numbers are not to be maintained too high. Internal training is compulsory. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand.	1	1	2	
4	Add 10 ml of DMEM (+) to stop the action of trypsin, then wash the flask/dish to wash off more cells. Transfer the cells into a 15 ml Falcon tube.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens,	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury	All personel handling cell lines are to have Hep B vaccination, and passage numbers are not to be maintained too high. Internal training is compulsory. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand.	1	1	2	

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5	Spin down cells by centrifuging at room temperature (25oC), 800 rpm for 5 minutes. After centrifuging, pour out the media without disturbing the cell pellet. Resuspend cell pellet with 1 ml DMEM (+) by pipetting up and down at least 10 – 15 times. Split cells into new culture dish/flask at the desired and recommended density (usually ~30-40% for cell maintenance).	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens, injury due to improper usage of centrifuge and fingers	Injury due to imbalanced centrifuge and trapping of limbs or fingers	Internal training is compulsory for centrifuge use and the centrifuge key. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand. When using the centrifuge, ensure centrifuge is balanced and rotor is placed correctly, and that all tubes are capped tightly. Close centrifuge properly and ensure that there are no funny sounds when centrifuge is running. Decontaminate waste using Presept tablets (dilute to 10% solution of activaed bleach to kill cells) and let the bleach decontaminate for half an hour before discarding with plenty of water and dilution.	1	1	2	