

Supplementary file 1: Cell Passage and Influenza Virus (non-H₂N₂) Inoculation

Operation Scoring Sheet (Total Score 100 Points)

Item	Assessment Content	Score	Deduction Criteria and Value		Deduction
Preparation	Preparation of materials for cell passage and virus inoculation.	6	Deduct 2 points for each missing item.	2	
	Dressing and entering the P2 laboratory conforms to biosafety.		Improper dressing, door not closed.	2	
	Selection of experimental equipment, reagents and specimens, and reasonable placement in the biosafety cabinet.		Reagents not checked, wrong placement order.	2	
Cell Observation	Observe cell growth and contamination in the bottle.	2	Not observed.	2	
Aseptic Operation	Disinfection of bottle/tube exterior, bottle (tube) mouth, pipette surface and operation area.	12	Missing one item (2 points per missing or incorrect item).	6	
	Aseptic operation while opening and closing bottle caps.		Bottle mouth not flame sterilized, cap placed improperly.	4	
	Aseptic operation during other steps.		Not paying attention to asepsis above the bottle mouth.	2	
Washing	Standard washing process.	8	Incorrect pouring direction, too much residue in the bottle.	2	
			Liquid added toward cell growth surface.	2	
			Incorrect washing liquid volume.	2	
			Not washed twice.	2	
Digestion	Standard digestion operation, accurate degree control.	6	Liquid added toward cell growth surface.	2	
			Washing time too long or short.	2	
			Incomplete or excessive digestion.	2	
Termination of Digestion and Dispersion	Add appropriate growth medium to terminate digestion in time.	6	Incorrect liquid amount.	2	
	Detach cells from the bottle wall.		Cells on the bottle wall not washed off.	2	
	Disperse cell clumps.		Cell clumps not dispersed by pipetting.	2	
Bottling	Evenly distribute cell suspension according to passage ratio.	4	Inaccurate distribution of cell suspension.	2	
	Add growth medium to suitable volume.		Incorrect growth medium volume.	2	
Virus Inoculation and Culture	Handling of influenza virus specimen.	12	Not handled according to SOP.	3	
	Inoculation of influenza virus specimen.		Not inoculated according to SOP.	3	
	Tighten bottle cap.		Cap not tightened.	2	
	Place cell bottle flat with cell growth surface down.		Not placed flat or misplaced.	2	

		Mark cell name, passage number, and date on the bottle surface.		Not marked or marked incorrectly (2 points for each error).	2	
Civilized Operation		Clean up workbench after operation, place items in designated positions (including chair).	4	Workbench not cleaned.	0.5	
				Items not placed as required, safety cabinet not cleaned/disinfected (2 points for each item).	1	
		Protect equipment.		Equipment damaged.	1	
		Restore pipette after us.		Not returned to maximum range after use.	1	
		Disinfect hands after experiment.		Hands not disinfected post-experiment, gloves not disposed of in trash.	0.5	
Biosafety Awareness		Safe, standard, with good biosafety awareness.	10	Consider bio-safety, standard operations, completion quality, etc.	10	
Aseptic Awareness		Proficient aseptic operation, good aseptic awareness.	10	Consider technical proficiency, operation quality, etc..	10	
Culture Result Scoring	Aseptic operation	No cell contamination.	20	Cell contamination.	5	
	Passage ratio	Accurate passage ratio.		Too many or too few cells.	5	
	Uniformity	Cells evenly distributed in the bottle.		Uneven cell distribution or cell clumps.	5	
	Cell vitality	Normal cell morphology and growth rate.		Abnormal morphology, slow growth.	5	
Total Score						