



## PLATED CELL CULTURE QUESTIONNAIRE

1. Were hands thoroughly washed and dried and were gloves worn before handling the flask containing plated cells?
2. Upon arrival, was the flask wiped with 70% ethanol before transferring to tissue culture hood?
3. Were cells processed in a clean tissue culture hood?
4. Upon arrival, was 100% of the supernatant removed and centrifuged?
5. What was the centrifuge speed and for what amount of time was the supernatant centrifuged?
6. Was cell viability determined using a manual or automated system?
7. What were the cells reconstituted in?
8. At what volume were the cells reconstituted in?
9. At what volume were the cells seeded in the flask?
10. Was the flask pre-coated with extracellular matrix?
11. After removing initial media from plated cells, was the media pre-warmed at 37°C prior to being added?
12. Was a water bath used to warm media to desired temperature?
13. If so, at what level by height was the media bottle submerged in the water bath and what was the temperature of the water bath?
14. How long was the media bottle in the water bath?
15. How much media was added to the flask?
16. Were the received cells incubated for 2 hours upon arrival?
17. After 2 hour incubation, was 100% media removed?
18. Were the attached cells washed with 1X PBS for 2-3 minutes?
19. Was 100% 1X PBS removed before adding trypsin to the attached cells?
20. Were cells allowed to trypsinize for 2-3 minutes?
21. Was trypsin neutralized after cells detached?
22. Were cells centrifuged at suggested speed and time?



23. Was supernatant discarded for the attached cells and cells reconstituted?
24. Once replated, were cells given 12-24 hours incubation to attach?
25. How often was the media changed?
26. Are you using any thing else for these cell cultures?
27. Are you using filter inserted pipette tips?
28. If yes did the tissue culture media come in contact with the filter?
29. Any other information that you may think would benefit us in identifying the root cause of the why the cells are not growing?