COMPARATIVE PERFORMANCE ANALYSIS OF CELL CULTURE FLASKS

CAROLINA COMPONENTS GROUP
Flow Through Us

919.635.8438
www.carolinaflow.com
customerservice@carolinaflow.com

VESSEL SELECTION

FlowLinX© culture flasks are used for successful growth and expansion of mammalian and microbial cells to study cell biology, test drug efficacy, develop vaccines and produce therapeutic proteins. The choice of a cell culture vessel can significantly impact cell behavior, growth kinetics and product yield. For example, the surface area, shape design and cleanliness can all affect cell culture performance. Flasks are commonly available in Erlenmeyer, multi-layer and roller bottle styles and can be integrated into sterile assemblies for use with various types of cell lines and equipment. Understanding the relative performance of the cell culture flask can help ensure success in your research, drug development or biopharmaceutical production.

The choice of a cell culture vessel can significantly impact cell behavior, growth kinetics and product yield.

CELL CULTURE STUDY

The study conducted provides a comprehensive examination of the comparative performance of various styles of cell culture flasks commonly used for cell cultivation. The study evaluated key parameters such as cell density and proliferation of CHO, HEK and bacterial cell lines in FlowLinX© cell culture flasks versus Corning© cell culture flasks. Additionally, the FlowLinX© cell culture flasks were tested for compliance with USP <788>, Particulate Matter in Injections.

Table 1 below highlights the styles of flasks chosen and tested.

FLASK	MANUFACTURER	MATERIAL OF CONSTRUCTION	WORKING VOLUME RANGE	BAFFLED
T-75	Carolina Components Group	Polystyrene	15-30 mL	N/A
125 mL Erlenmeyer	Carolina Components Group	Polycarbonate	30-50 mL	Yes
125 mL Erlenmeyer	Carolina Components Group	PETG	30-50 mL	No
500 mL Erlenmeyer	Carolina Components Group	Polycarbonate	125-160 mL	Yes
500 mL Erlenmeyer	Carolina Components Group	PETG	125-160 mL	No
Cell Stack	Carolina Components Group	Polystyrene	200-500 mL	N/A
T-75	Corning©	Polystyrene	15-30 mL	N/A
125 mL Erlenmeyer	Corning©	Polycarbonate	30-50 mL	Yes
500 mL Erlenmeyer	Corning©	Polycarbonate	125-160 mL	Yes
Cell Stack	Corning©	Polystyrene	200-500 mL	N/A

A comprehensive examination of the comparative performance of various styles of cell culture flasks commonly used for cell cultivation.

CHO CULTURE PERFORMANCE STUDY:

CHO-K1 cells were obtained from ATCC and thawed following the ATCC guidance in a water bath. The cells were suspended in CHO complete growth media (Innovative Research) and spun down at 125xg in a centrifuge. The pellet was resuspended in 2 mL of media and dispensed into 13mL of prewarmed media in a FlowLinX© and Corning© T-75 flask. The culture was placed into a 37°C incubator at 5% CO_2 and fed every 3 days until the target confluence was observed. On day 6 the cells were passaged into CCG and Corning© 125mL Erlenmeyer flasks and allowed to grow until the confluence target was met. On day 12 the cells were passaged into CCG and Corning© 500 mL Erlenmeyer flasks and allowed to grow until the confluence target was met. On day 17 the cells were passaged into CCG and Corning© multi-layer cell stacks and allowed to grow until the confluence target was met. As part of the analysis, all spent media was collected and monitored for LDH and IGG on a Cedex Bioanalyzer.

MANUFACTURER	MATERIAL	FLASK	CULTURE DAY	CULTURE VOLUME	LDH	IGG	% CONFLUENCE	CELL DENSITY (CELLS/ML)
CCG and Corning©	PS/PS/PETG	T-75	0	25	51.82	0.054	0	1.0E04
Corning©	PS	T-75	3	25	48.93	0.052	20	2.4E5
CCG	PS	T-75	3	25	41.83	0.053	20	2.5E5
Corning©	PS	T-75	5	25	43.98	0.133	25	1.2E6
CCG	PS	T-75	5	25	44.87	0.122	25	1.3E6
Corning©	PS	T-75	6	25	51.53	0.202	40	3.7E6
CCG	PS	T-75	6	25	52.77	0.207	35	3.5E6
Corning©	PS	125mL Flask	6	30	58.89	0.102	0	1.0E4
CCG	PS	125mL Flask	6	30	60.12	0.112	0	1.0E4
CCG	PETG	125mL Flask	6	30	60.12	0.099	0	1.0E4
Corning©	PS	125mL Flask	8	30	62.73	0.255	30	3.7E5
CCG	PS	125mL Flask	8	30	62.24	0.262	30	3.5E5
CCG	PETG	125mL Flask	8	30	62.24	0.262	35	3.5E5
Corning©	PS	125mL Flask	10	30	66.73	0.345	30	2.9E6

Table continues on next page.

MANUFACTURER	MATERIAL	FLASK	CULTURE DAY	CULTURE VOLUME	LDH	IGG	% CONFLUENCE	CELL DENSITY (CELLS/ML)
CCG	PS	125mL Flask	10	30	67.24	0.332	30	2.7E6
CCG	PETG	125mL Flask	10	30	66.24	0.323	25	2.8E6
Corning©	PS	500mL Flask	12	125	46.23	0.172	0	1.0E4
CCG	PS	500mL Flask	12	125	49.77	0.181	0	1.0E4
CCG	PETG	500mL Flask	12	125	47.11	0.178	0	1.0E4
Corning©	PS	500mL Flask	14	125	46.23	0.263	20	4.2E5
CCG	PS	500mL Flask	14	125	49.77	0.277	20	4.7E5
CCG	PETG	500mL Flask	14	125	47.11	0.271	20	4.4E5
Corning©	PS	500mL Flask	16	125	51.23	0.555	40	3.1E6
CCG	PS	500mL Flask	16	125	52.63	0.534	40	2.8E6
CCG	PETG	500mL Flask	16	125	51.12	0549	35	3.0E6
Corning©	PS	Cell Stack	17	300	32.23	0.234	0	1.0E4
CCG	PS	Cell Stack	17	300	33.63	0.222	0	1.0E4
Corning©	PS	Cell Stack	19	300	44.23	0.355	20	2.9E5
CCG	PS	Cell Stack	19	300	44.63	0.434	25	2.7E5
Corning©	PS	Cell Stack	21	300	48.13	0.515	40	3.2E6
CCG	PS	Cell Stack	21	300	49.88	0.562	40	3.4E6
Corning©	PS	Cell Stack	23	300	52.76	0.515	40	5.2E6
CCG	PS	Cell Stack	23	300	54.51	0.562	40	5.0E6

HEK CULTURE PERFORMANCE STUDY:

HEK293 cells were obtained from ATCC and thawed following the ATCC guidance in a water bath. The cells were suspended in HEK293 complete growth media and spun down at 125xg in a centrifuge. The pellet was resuspended in 4 mL of media and dispensed into 13mL of prewarmed media in CCG and Corning© T-75 flasks. The culture was placed into a 37°C incubator at 5% CO_2 and fed every 3 days until the target confluence was observed. On day 6 the cells were passaged into CCG and Corning© 125mL Erlenmeyer flasks and allowed to grow until the confluence target was met. On day 12 the cells were passaged into CCG and Corning© 500 mL Erlenmeyer flasks and allowed to grow until the confluence target was met. On day 16 the cells were passaged into CCG and Corning© multi-layer cell stacks and allowed to grow until the confluence target was met. As part of the analysis, all spent media was collected and monitored for LDH and IGG on a Cedex Bioanalyzer.

The below table outlines the results of the study.

MANUFACTURER	MATERIAL	FLASK	CULTURE DAY	CULTURE VOLUME	LDH	IGG	% CONFLUENCE	CELL DENSITY (CELLS/ML)
Corning©	PS	T-75	0	25	47.86	0.052	0	4.3E4
CCG	PS	T-75	0	25	51.97	0.052	0	4.3E4
Corning©	PS	T-75	3	25	47.14	0.064	20	6.6E5
CCG	PS	T-75	3	25	41.17	0.062	30	6.7E5
Corning©	PS	T-75	5	25	43.68	0.102	20	2.6E6
CCG	PS	T-75	5	25	48.33	0.113	30	2.2E6
Corning©	PC	125mL Flask	6	30	40.18	0.039	0	4.3E4
CCG	PC	125mL Flask	6	30	40.61	0.032	0	4.3E4
CCG	PETG	125mL Flask	6	30	39.50	0.034	0	4.3E4
Corning©	PC	125mL Flask	7	30	38.08	0.122	20	5.7E5
CCG	PC	125mL Flask	7	30	38.69	0.132	20	5.9E5
CCG	PETG	125mL Flask	7	30	39.77	0.128	20	6.0E5
Corning©	PC	125mL Flask	9	30	39.65	0.423	45	3.2E6
CCG	PC	125mL Flask	9	30	40.23	0.444	50	3.2E6

Table continues on next page.

MANUFACTURER	MATERIAL	FLASK	CULTURE DAY	CULTURE VOLUME	LDH	IGG	% CONFLUENCE	CELL DENSITY (CELLS/ML)
CCG	PETG	125mL Flask	9	30	39.57	0.459	50	2.9E6
Corning©	PS	500mL Flask	11	125	37.65	0.226	0	4.3E4
CCG	PS	500mL Flask	11	125	34.92	0.221	0	4.3E4
CCG	PETG	500mL Flask	11	125	36.22	0.235	0	4.3E4
Corning©	PC	500mL Flask	13	125	40.11	0.337	30	6.8E5
CCG	PC	500mL Flask	13	125	41.48	0.354	35	6.3E5
CCG	PETG	500mL Flask	13	125	42.49	0.362	30	7.1E5
Corning©	PC	500mL Flask	15	125	N/A	0.467	40	4.1E6
CCG	PC	500mL Flask	15	125	40.1	0.510	45	4.3E6
CCG	PETG	500mL Flask	15	125	42.49	0.488	50	4.1E6
Corning©	PS	Cell Stack	16	300	42.76	0.234	0	4.3E4
CCG	PS	Cell Stack	16	300	44.51	0.254	0	4.3E4
Corning©	PS	Cell Stack	18	300	48.97	0.555	35	5.2E5
CCG	PS	Cell Stack	18	300	49.39	0.562	40	5.0E5
Corning©	PS	Cell Stack	20	300	52.76	0.621	60	2.7E6
CCG	PS	Cell Stack	20	300	54.51	0.644	60	3.0E6
Corning©	PS	Cell Stack	22	300	58.02	0.898	60	4.8E6
CCG	PS	Cell Stack	22	300	59.32	0.920	60	5.1E6

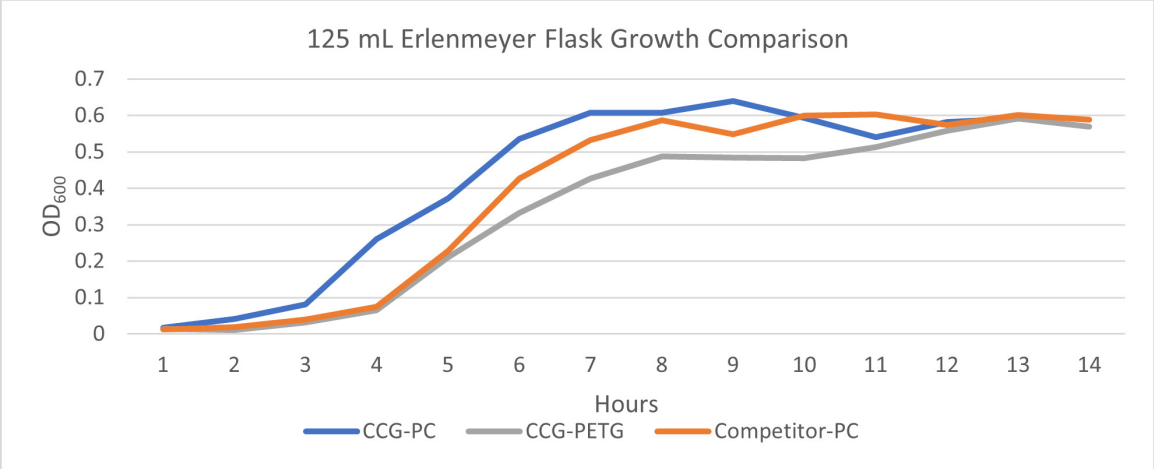
BACTERIAL CULTURE PERFORMANCE STUDY:

Bacterial culture, *Staphylococcus aureus*, from ATCC was used to inoculate each type of vessel with BHI Hi-Veg media broth. Optical density at 600nm was used to track the growth performance of each culture vessel over a 12-hour period. All cultures were incubated at 37°C and flask type vessels were set to agitate at 100 rpm while flat style vessels were set to 50 rpm. Samples of 100 µL were removed at each time point tested to minimize the cumulative effect of sample removal on overall culture volume. All flasks were seeded with the same starter culture of *Staphylococcus aureus* at 9.30e8 CFU/mL at 1% of the total media volume.

The following tables and graphs display the growth performance of *Staphylococcus aureus* as measured by OD_{600} over time. T-0 refers to the time point directly before inoculation and T+0 refers to the time point directly after inoculation. Tracking continues for 12 hours, sufficient to show growth phases transition out of exponential and into stationary and decline phases.

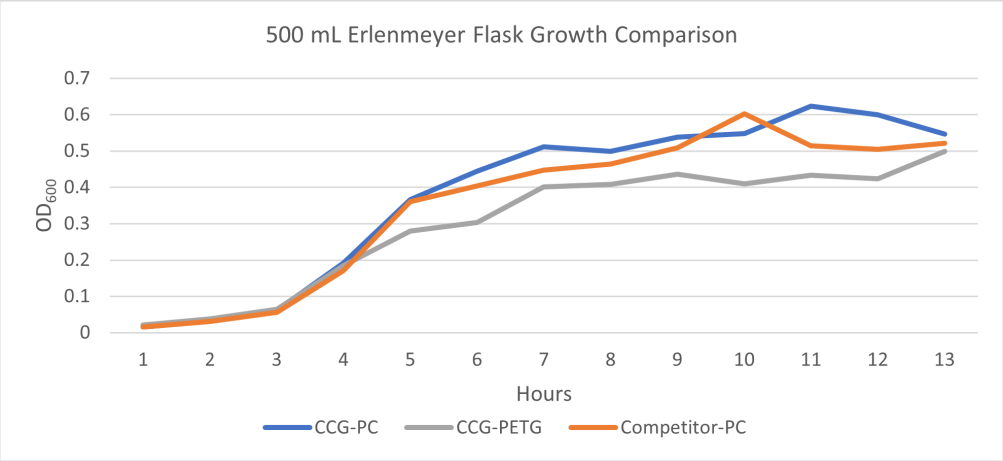
125 ML SHAKE FLASK COMPARISON:

	OD_{600}													
VESSEL TYPE	T-0	T+0	1 HR	2 HR	3 HR	4 HR	5 HR	6 HR	7 HR	8 HR	9 HR	10 HR	11 HR	12 HR
FlowLinX© PC 125mL Flask	0.013	0.017	0.041	0.082	0.26	0.373	0.535	0.607	0.607	0.640	0.593	0.540	0.582	0.592
FlowLinX© PETG 125mL Flask	0.014	0.011	0.031	0.064	0.211	0.332	0.426	0.487	0.485	0.482	0.513	0.558	0.591	0.569
Corning© PC 125mL Flask	0.013	0.019	0.040	0.075	0.229	0.427	0.532	0.587	0.549	0.600	0.603	0.574	0.601	0.588



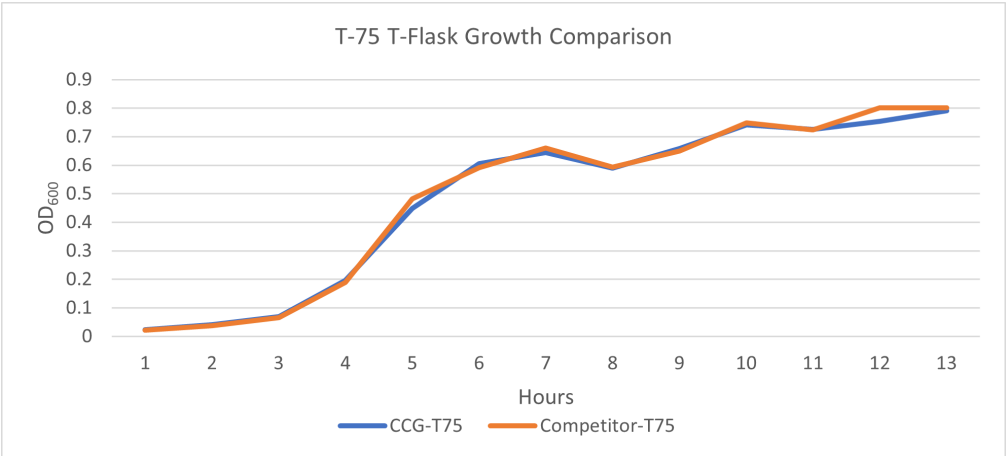
500 ML ERLENMEYER FLASK COMPARISON:

	OD_{600}													
VESSEL TYPE	T-0	T+0	1 HR	2 HR	3 HR	4 HR	5 HR	6 HR	7 HR	8 HR	9 HR	10 HR	11 HR	12 HR
FlowLinX© PC 500mL Flask	0.013	0.015	0.033	0.062	0.192	0.367	0.445	0.511	0.499	0.538	0.548	0.624	0.600	0.547
FlowLinX© PETG 500mL Flask	0.013	0.021	0.037	0.065	0.185	0.279	0.303	0.401	0.408	0.436	0.41	0.433	0.424	0.499
Corning© PC 500mL Flask	0.014	0.015	0.031	0.056	0.17	0.361	0.404	0.447	0.464	0.509	0.603	0.514	0.505	0.521



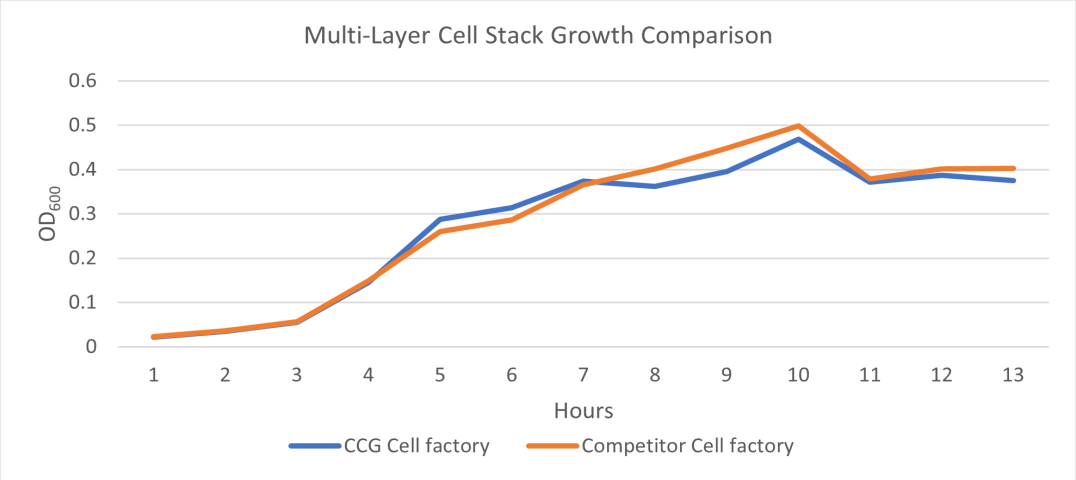
T-75 FLAT CULTURE FLASK GROWTH COMPARISON:

	OD_{600}													
VESSEL TYPE	T-0	T+0	1 HR	2 HR	3 HR	4 HR	5 HR	6 HR	7 HR	8 HR	9 HR	10 HR	11 HR	12 HR
CCG T75 T-Flask	0.014	0.023	0.040	0.069	0.196	0.449	0.605	0.645	0.59	0.659	0.742	0.725	0.754	0.791
Corning© T75 T- Flask	0.013	0.021	0.038	0.066	0.19	0.483	0.591	0.661	0.594	0.65	0.748	0.724	0.801	0.802



CELL FACTORY FLAT CULTURE FLASK GROWTH COMPARISON:

	OD_{600}													
VESSEL TYPE	T-0	T+0	1 HR	2 HR	3 HR	4 HR	5 HR	6 HR	7 HR	8 HR	9 HR	10 HR	11 HR	12 HR
CCG Cell Stack	0.013	0.022	0.034	0.055	0.145	0.288	0.314	0.374	0.362	0.396	0.469	0.371	0.387	0.375
Corning© Cell Stack	0.013	0.023	0.036	0.057	0.149	0.26	0.287	0.365	0.401	0.448	0.498	0.379	0.401	0.403



USP <788> TESTING OF FLOWLINX®CELL CULTURE FLASK

FLASK DESCRIPTION	PARTICULATES >10 µM	PARTICULATES > 25 µM	MEETS USP<788>
T-75	0	0	Yes
125mL PC Erlenmeyer	0	0	Yes
125mL PETG Erlenmeyer	0	0	Yes
500mL PC Erlenmeyer	0	0	Yes
500mL PETG Erlenmeyer	0	0	Yes
Multi-Layer Cell Stack	0	0	Yes

STUDY CONCLUSION:

Results of the study demonstrate equivalent performance of FlowLinX© culture flasks in mammalian cell cultures and bacterial fermentation when compared to comparable Corning© culture flasks. Organizations can successfully implement FlowLinX© culture flasks for their research, drug development and biopharmaceutical production needs.

Testing was performed by bioX LLC (bioxeng.com) at their Bioprocess Applications Testing Laboratory in Salem, NH. bioX is an independent third-party specializing in single use materials and equipment testing intended for use in cGMP Manufacturing. All data was generated under controlled laboratory conditions in compliance with a quality management system utilizing NIST traceable measurement devices and standards.