Rapid Contamination Detection in Mammalian Cell Cultures

Why Should You Care?

Mammalian cells are at the heart of biotechnology, powering the production of life-saving biopharmaceuticals, vaccines, and proteins.

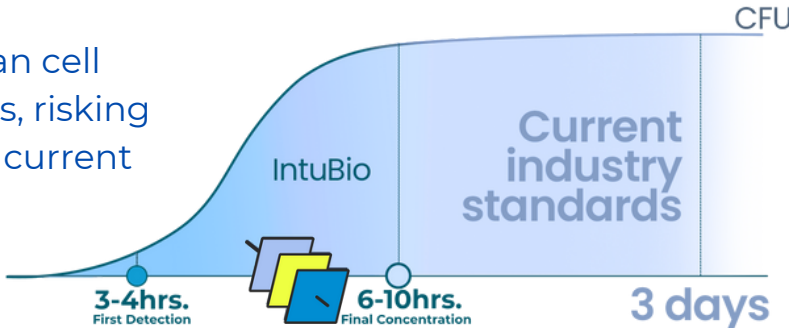
The global mammalian cell culture market is projected to exceed **$57 billion by 2029**, with ongoing research optimizing CHO and HEK293 cell lines for large-scale use(1), optimizing contamination detection is more critical than ever!

Traditional methods take days

But what if we told you that IntuGrow can do it in just **3 hours.**

What's the Challenge?

Contaminants in mammalian cell cultures lead to costly delays, risking entire production lines. The current methods are too slow.



IntuGrow streamlines this process, reducing detection time to just **3 hours** through high-resolution scanning and microscopy. This protocol is developed by analyzing mammalian cell culture matrices and adapting standard procedures for efficient contamination detection.

The Experiment

Develop a simple yet effective protocol using IntuGrow to detect bacterial contamination in mammalian cell cultures (CHO & HEK293).

How Fast Were the Results?

Time to Detect (TTD) was consistently **3 hours** for all samples.

Time to Result (TTR) varied with the fastest TTR being 5 hours.

Key Process

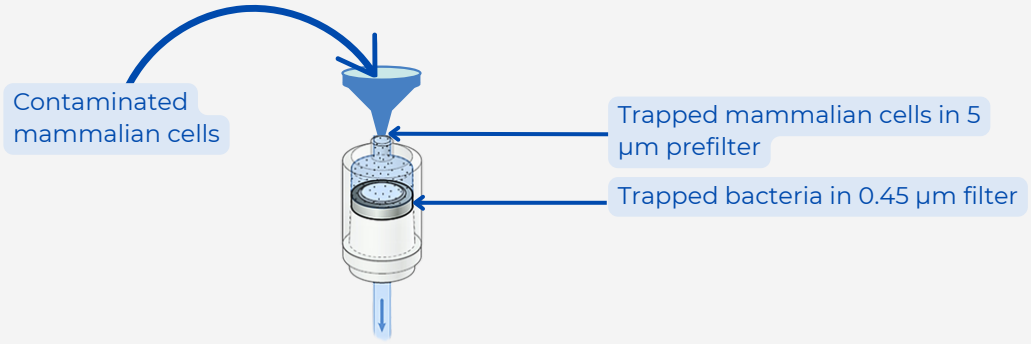
1 Contaminating the Cultures

We deliberately contaminated HEK and CHO cell cultures using a skin swab.

2 Separating the Cells

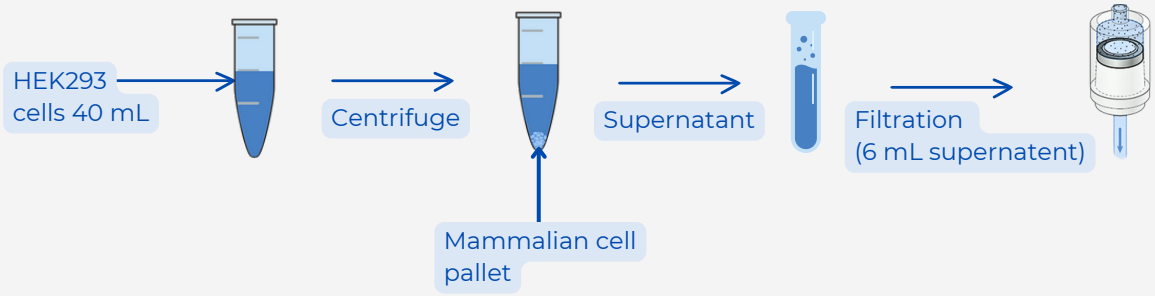
Two approaches tested: Prefiltration and Centrifugation

Prefiltration (Primary approach)



Centrifugation

Gentle spinning at 200 g for 5 min to pellet mammalian cells while leaving bacteria behind.



3 Capturing the Culprits

We used IntuGrow filters (0.45 µm PTFE) to trap bacteria The filters were scanned every hour for 24 hrs. using high-resolution scanning.

Results that matter

Both prefiltration and centrifugation effectively removed mammalian cells.

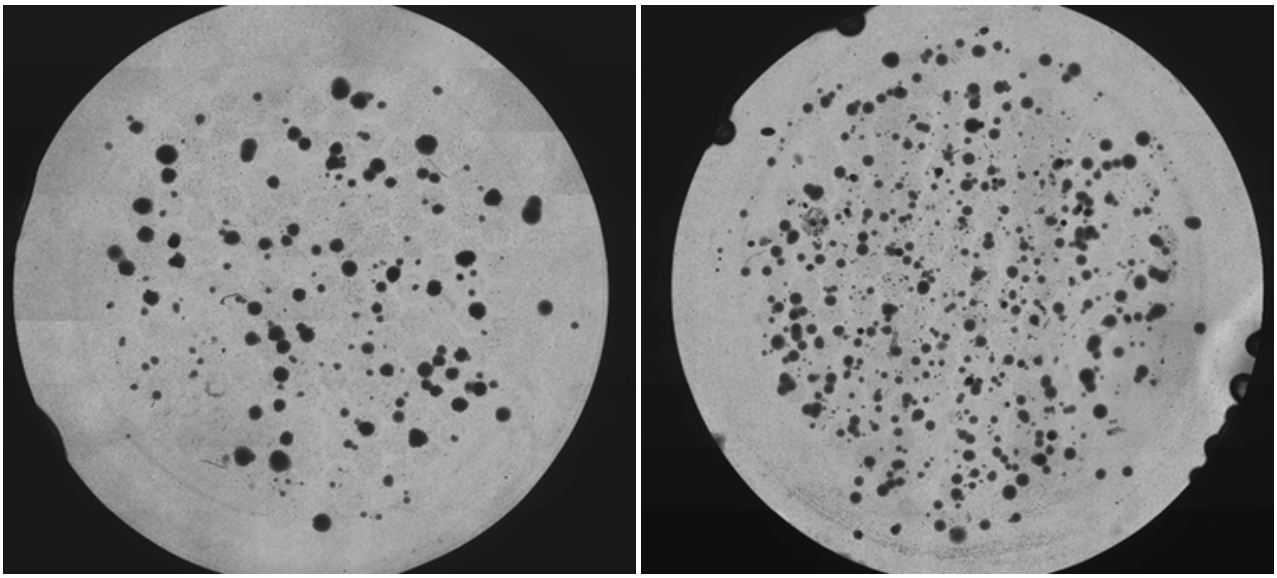


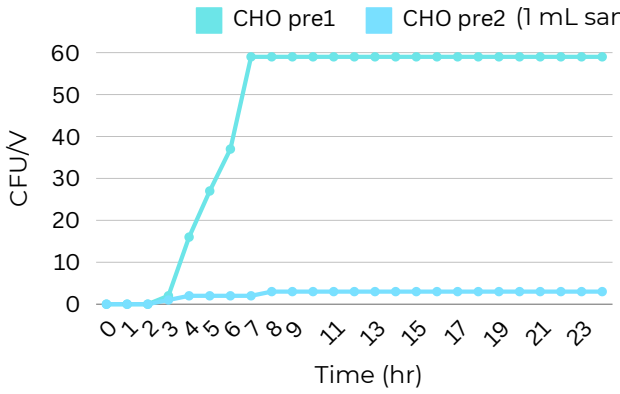
Figure 1. (left) HEK293 prefilter 1, 5ml. Visible CFUs and absence of mammalian cells. Some debris is also visible. (right) Supernatant. Higher CFU count due to the higher volume of analyte (6 mL). More debris can be seen in the background. (see table 1)

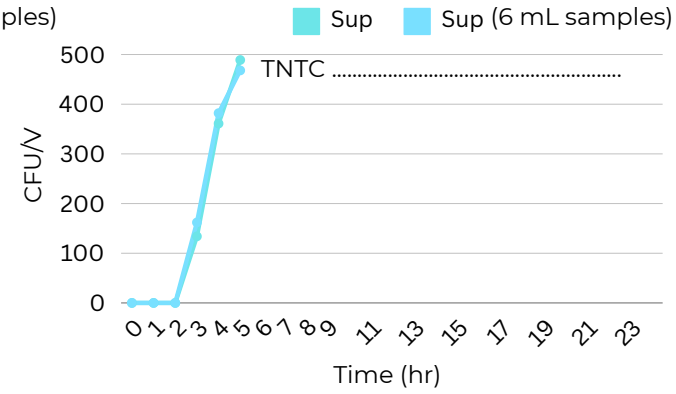
Time to Detect (TTD) was consistently 3 hours for all samples. Time to Result (TTR) varied with the fastest TTR being 5 hours. (see table 1).

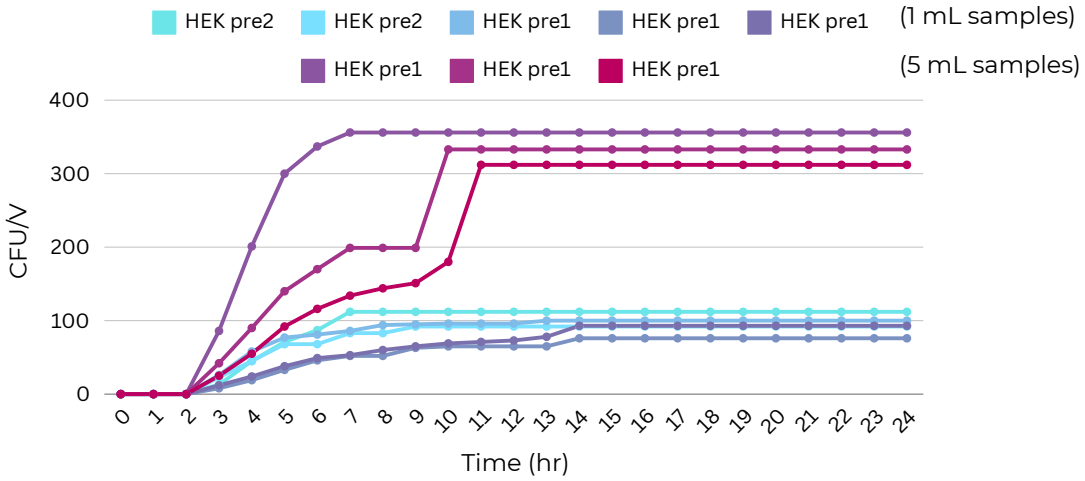
Sample Name	Analyte Volume (ml)	TTD (hr)	TTR (hr)
HEK prefilter 2	1	3	7
HEK prefilter 2	1	3	6
HEK prefilter 1	1	3	7
HEK prefilter 1	1	3	14
HEK prefilter 1	1	3	14
HEK prefilter 1	5	3	7
HEK prefilter 1	5	3	10
HEK prefilter 1	5	3	11
CHO prefilter 2	5	3	8
CHO prefilter 1	5	3	7
Supernatant	6	3	5 (TNTC)*
Supernatant	6	3	5 (TNTC)*

*Table 1. TTD and TTR of all the samples analyzed. TNTC= Too Numerous To Count. * Here, TTR refers to the timestamp the analyzer flagged for TNTC.*

All samples were scanned every hour to monitor for any bacterial growth. After 3 hours, at least one colony-forming unit (CFU) was detected in all samples, as shown in the graphs below.







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Conclusions

- CFU counts scaled predictably with sample volume, proving method suitability.
- Prefiltration effectively removed mammalian cells, allowing only bacteria to pass.
- Filtration & analysis setup is reliable for up to 5 mL samples.
- Supernatant filtration after gentle centrifugation produced comparable results to prefiltration, making it a viable alternative.

Methods and Materials

Two different cell types were used, namely CHO and HEK293 cell cultures. Cell count was approximately 2 x 10^6 cells/mL for both cultures. The cells were cultivated at 37°C, 5% CO2 with shaking at 130 rpm. For CHO cells, Gibco™ CD CHO Medium was used (Thermo Fisher Scientific, #10743029), pre-optimized with 8 mM L-glutamine (Thermo Fisher Scientific, #A291680), and supplemented with Antibiotic-Antimycotic (100X) Gibco™ (Thermo Fisher Scientific, #15240062). For the HEK293 cells: HyCell TransFx-H (Nordic Biolabs, #SH30939.02) + 4mM Glutamax Gibco™ (Thermo Fisher Scientific, #35050061) + 0.1% Pluronic F-68 Gibco™ (Thermo Fisher Scientific , #24040032).

Four flasks, 15 mL each, of HEK293 cell culture flasks were mixed, and a skin swab was used to contaminate the total volume (60 mL) of the cultures. A skin swab was also used to contaminate the CHO culture (15 mL). The analysis took place 30 minutes after the contamination.

Two different pre-filters were tested to remove mammalian cells: prefilter 1
(Material: Hydrophilic PVDF, Pore Size: 5 µm, Diameter: 25 mm) and prefilter 2 (Material: Cellulose Acetate, Pore size: 5 µm, diameter, 25 mm).

After utilizing the prefiltration step, centrifugation was also used on the remaining HEK293 cell culture (40 mL) to separate growth media from mammalian cells. Caution was applied to only pellet the cells and not the bacteria, using very gentle centrifugation conditions, namely: 200 g for 5 min.

Removing the pre-filtration step offers an advantage when working with larger sample volumes since 6 mL were filtered without any clogging of the 0.45 µm filter, suggesting minimal presence of large cells or debris. While this approach improves efficiency, it is worth noting that recovery rates were not accounted for.

Following the separation, IntuGrow filters were used (IB.02.0000. Material: PTFE, Pore size: 0.45 µm) to capture potential bacteria.

References

1. Ltd, R. a. M. (n.d.-b). Cell Culture Market Size, Competitors & Forecast to 2029. Research and Markets Ltd 2025. https://www.researchandmarkets.com/report/cell-culture#src-pos-1