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Application Note

Knowing Your Baseline

How Baseline Studies Can Ensure Successful Virus Filtration Spiking Studies

Demonstrating the viral clearance capabilities of specific unit operations in downstream processing is a key component of regulatory submissions for biopharmaceuticals. While most virus filters provide robust viral clearance across a broad range of conditions, there are numerous artifacts that can limit throughput on filters such as the Viresolve® Pro Device.[1]

A baseline run uses the process fluid, in the absence of virus, to confirm the throughput performance on the Viresolve® Pro Device.

Why Perform a Baseline Run?

Conducting a baseline run, and evaluating the throughput performance of the process fluid prior to virus testing, increases the likelihood of achieving both throughput and retention targets on the virus filter. Data from a baseline run used as a scale-down model for viral clearance studies should be comparable to the full-scale manufacturing process.[2]

However, throughput performance of the process fluid during small-scale viral clearance studies can be affected by artifacts not normally observed in process development or manufacturing: different fluid concentrations, process fluid age, freeze/thaw, agitation, adsorptive prefilter decoupling, among others.[1] Artifacts of viral clearance studies that lower throughput, can increase filtration area requirements and production costs in manufacturing.

Information from a baseline run provides guidance for corrective actions that may be needed before viral clearance studies begin.

Baseline throughput data should look like previous data

Baseline throughput data should capture the volume of fluid filtered over time, either manually or with a data acquisition system. Often less detailed information like processing time or average flux is available and sufficient to confirm performance is within the expected range. Previous data for the same process fluid may be generated during:

- Process development runs
  - Performance with frozen/thawed process fluid
  - Prefilter decoupling
  - Runs using the same lot of process fluid
- Pilot, engineering, and manufacturing runs
- Previous viral clearance runs
  - The same or similar process conditions
  - The same virus filter

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Leveraging a Baseline Run to Optimize Clearance Studies.

Procedures for the baseline run should mimic those of the spiked runs as closely as possible and throughput targets should be the same as the spiked runs. Truncated runs, with small fluid volumes, can provide useful information but can't predict unusual flow decay profiles at higher throughputs.

In contrast to the manufacturing process, spiking studies typically decouple the prefilter and virus filter, **Figure 1A**. The process fluid is filtered across the prefilter, spiked with virus, microfiltered to remove viral aggregates and then the spiked fluid is processed over the virus filter. This traditional approach maximizes potential viral clearance claims on the virus filter by eliminating the possibility of virus removal on the prefilter.

By contrast, in process development and manufacturing, the prefilter and virus filter are typically coupled, **Figure 1B**.

Once the baseline run is completed, throughput data (L/m^2) should be compared to previous data. Dependent on the results, different corrective actions may be needed, **Table 1**.

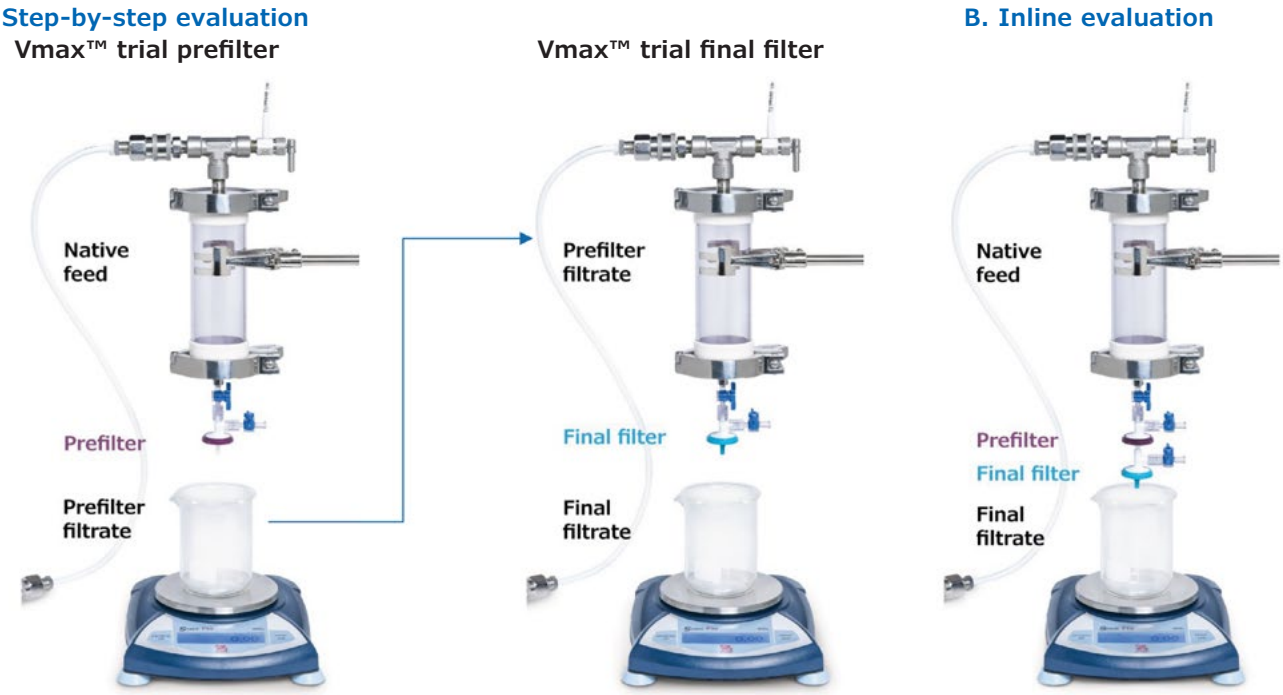

Figure 1. **(A)** Adsorptive prefilter decoupled from virus filter **(B)** adsorptive prefilter coupled with virus filter

Table 1. Potential corrective actions if baseline throughput results are different to previous throughput data.

Baseline results	Actions needed	Possible corrective actions
Baseline flow decay is in line (~10-15%) with previous results	No changes needed in handling or operational procedures	
Baseline flow decay doesn't match previous data (> 20% difference)	Adjust procedures to improve comparability with previous results	• Use freeze thaw conditions from process development • Minimize agitation during mixing • Use dip tubes for collecting prefiltered material, minimize time between process steps, pour gently and minimize air-liquid interface • Perform vacuum filtration slowly or filter under constant pressure
High flow decay (>60%) in both previous and current results	Corrective actions from above plus adjustments in operational procedures	• Adjust throughput targets • Adjust virus spike level • Adjust operational or handling procedures to minimize flow decay: inline injection, offline monodispersity checks

Decoupling the Prefilter and Virus Filter may Impact Throughput

Decoupling the adsorptive prefilter from Viresolve® Pro Device introduces material transfers, manipulations and time between the prefiltration and virus filtration step. These procedures are not typical of the large-scale manufacturing process and may increase aggregate formation in the process fluid leading to premature fouling of the virus filter.

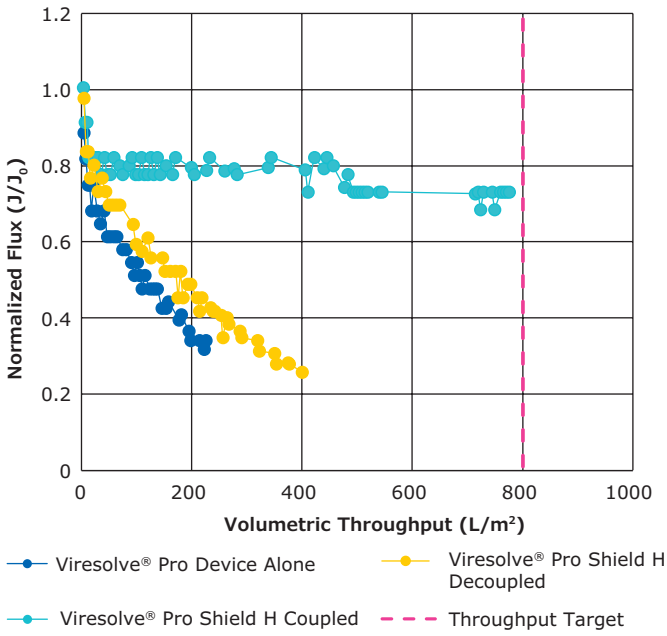

Figure 2. Decoupling prefilter and virus filter can reduce throughput.

Figure 2 shows the impact of decoupling the Viresolve® Pro Shield H prefilter from the Viresolve® Pro Device: normalized flux was reduced by ~60%. Manipulations of the process fluid resulted in high flow decay and the throughput target was not reached.

Mitigating these impacts could be accomplished by:

- Gentler handling procedures such as siphoning to reduce air-liquid interface
- Coupling the prefilter-virus filter and modifying spiking procedures to in-line injection

Gentle Handling Can Mitigate Aggregate Formation

Air-liquid interfaces are a common source of aggregate formation during virus filter clearance studies, especially after adsorptive prefiltration. Gentle pouring, minimizing splashing during vacuum microfiltration, and using dip tubes on filter outlets can mitigate the negative impact of aggregates. Sometimes these steps aren't enough and additional steps may be needed such as:

- Gravity siphoning: instead of pouring, use a tube to transfer the process fluid from one vessel to another (**Figure 3A**).
- Replace vacuum microfiltration: instead of using a vacuum filter for microfiltration of virus spiked fluid, perform microfiltration with OptiScale® 25 filters at 10-15 psi constant pressure with a dip tube on the filter outlet (**Figure 3B**).
- Eliminate microfiltration of the entire process fluid and perform offline assessment of viral monodispersity using a small representative sample (**Figure 3C**).

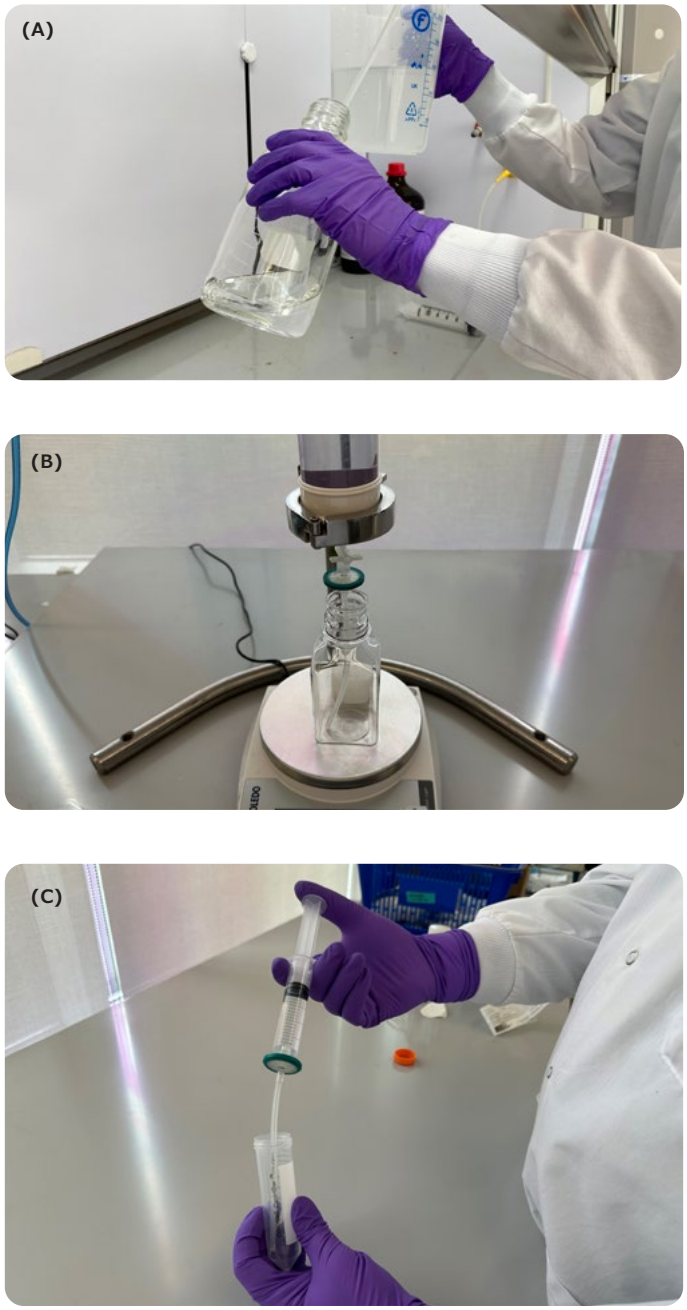

Figure 3. Gentle handling techniques: **(A)** gravity siphoning, **(B)** filtration with constant pressure, and **(C)** microfiltration for monodispersity assessment on a small offline sample.

The benefits of careful handling on throughput of spiked runs is shown in **Figure 4**. The initial baseline run with process fluid for the spiking study had ~ 70% flow decay. By siphoning process fluid during transfer and adopting offline microfiltration, filter fouling was reduced resulting in ~25% flow decay at the target throughput.

If the throughput of the baseline run cannot be improved using these approaches, then in-line injection should be assessed.

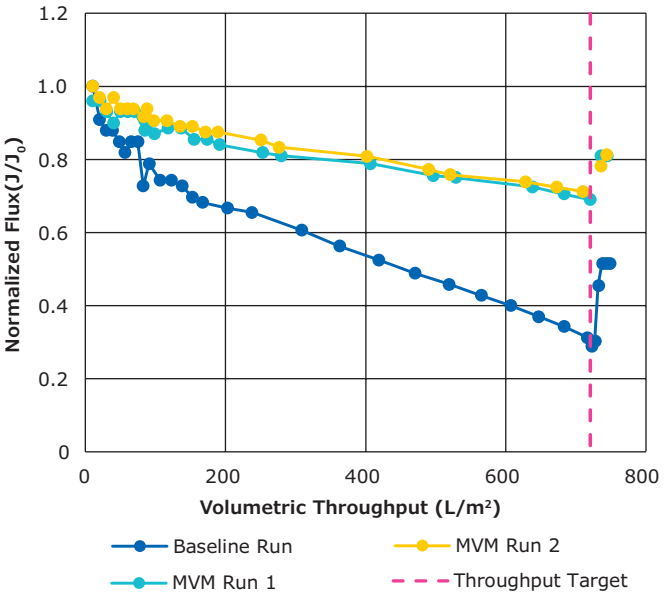

Figure 4. Spiked run flow decay improved through gentle handling and corrective actions.

Monodispersity Checks

During viral clearance validation studies, it is important to ensure that the virus particles are not aggregated.[2,4,5] Aggregated virus particles are larger than monodispersed viral particles and therefore easier for the virus filter to remove. High levels of aggregated virus could result in overestimating the virus removal capabilities of the filter. To assess the levels of aggregated virus in spiked process fluid, the fluid is sampled before and after filtration over a 0.1 - 0.45 µm (depending on virus size) filter, typically a bottle-top vacuum filter. This test is often referred to as the monodispersity check.

In-line Injection

In-line injection enables addition of a virus spike directly into the process fluid as it passes between a coupled prefilter and virus filter.[3] This method eliminates air-liquid interfaces between the prefilter and the virus filter and reduces the likelihood of aggregate or foulant formation. Virus is added to the process fluid using a syringe pump with the flow rate adjusted dependent on the flow rate of the Viresolve® Pro Device, **Figure 5**. Although this method offers advantages for reaching throughput targets, the setup, control and data recording is more complex than standard spiking approaches and should only be used when other options are not possible.

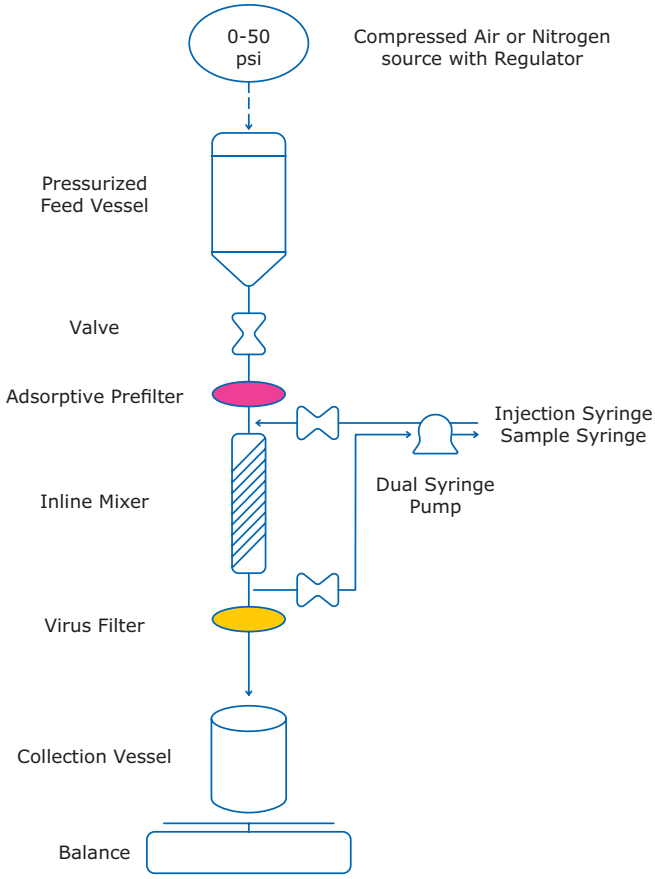

Figure 5. In-line Virus Spiking Set-up

Freeze/Thaw Effects on Throughput

Viral clearance studies often use process fluid that has been frozen at -20 or -80 °C during shipment to the testing facility. **Figure 6** illustrates the impact that freeze/thaw can have on throughput performance.

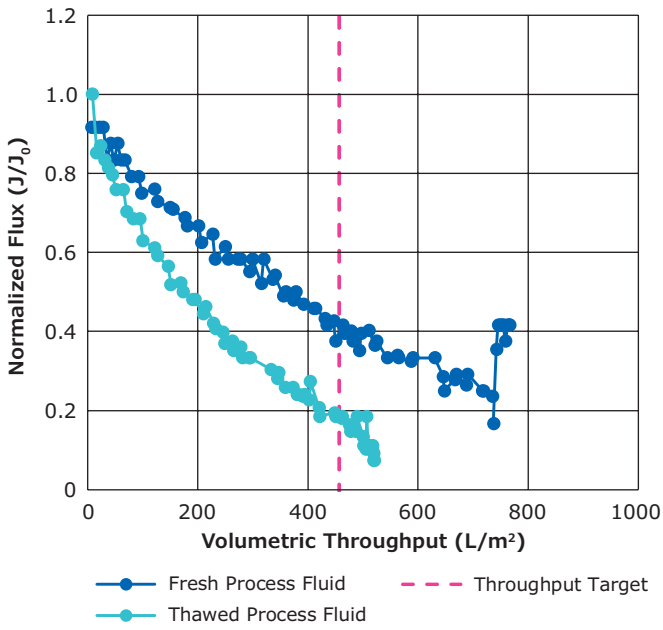

Figure 6. Throughput differences between fresh and freeze/thawed process fluid

In the cases where acceptable freeze/thaw throughput data is available, it is important to replicate the thawing procedure to optimize filtration throughput. Where throughput of freeze/thawed process fluid is not representative of fresh material, restoring the process fluid performance may be justified.

Restoring Process Fluid Performance

Restoring filtration performance of freeze/thawed process fluid to that of fresh material for viral clearance studies can be accomplished using different approaches:

- sterile filtration of process fluid after thawing
- increasing prefiltration area
- adding an adsorptive prefilter for process fluid restoration

Figure 7 shows an example of how sterile filtration after thawing can improve process fluid throughput. The larger pore size of the sterilizing membrane filter removes large aggregates or foulants that plug the virus filter.

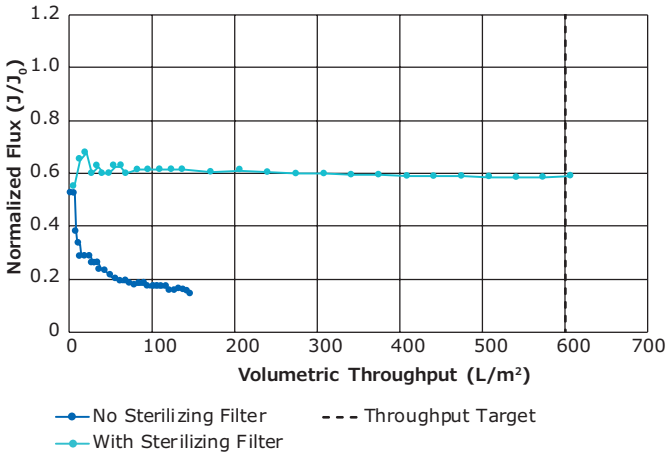

Figure 7. Sterile filtration of process fluid improves throughput

In many cases, sterile filtration alone will not mitigate the effects of freeze/thaw or shipping, and adsorptive prefiltration may be needed. **Figure 8** shows results of a study where the baseline performance (Blue) was significantly worse than in process development (Yellow). A repeat baseline (Cyan) with the Viresolve® Pro Shield prefilter in-line with the Viresolve® Pro Device showed minimal improvement; the Viresolve® Pro Shield prefilter was the prefilter specified for this manufacturing process. These results suggested that gentle handling procedures would not improve performance to meet the desired throughput. When the process fluid was prefiltered over the Viresolve® Prefilter, throughput performance was restored (Magenta).

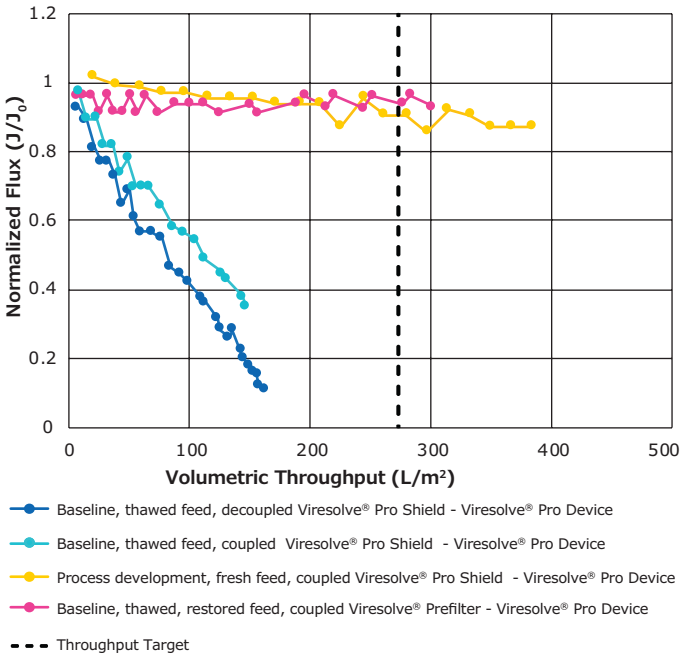

Figure 8. Addition of Viresolve® Prefilter to mitigate the impact of freeze-thaw and restore filtration throughput performance

Prefilter substitutions like this or prefilter area changes during viral clearance studies may be justified if throughput data is not representative of the manufacturing process and modifying the prefiltration step results in a more representative process fluid.[1] If this approach was not adopted, either fresh process fluid would need to be generated for the viral clearance study or the virus filter throughput lowered which would increase filtration area requirements and production costs in manufacturing.

Confirming the Virus Spike Does Not Impact Throughput

If time and material is available, many manufacturers opt to confirm the throughput performance of the process fluid on Viresolve® Pro Devices in the presence of a virus spike.

- **Scoping runs:** process fluid spiked with virus, run under process conditions and provide representative performance of the spiked runs. Typically samples are not collected for assaying.
- **Mock-spike runs:** process fluid spiked with virus suspension buffer. Provides insights on potential interactions between the buffer and process fluid; these tend to be less informative than spiked runs.

It is important to note that scoping or mock-spike runs should be performed in addition to, not in place of, a baseline run. Without a baseline run it is difficult to clarify if any negative impacts on throughput are due to an artifact of the viral clearance study or the virus spike addition.

Spike Percentage Recommendations

During viral clearance studies, it is important to balance the quantity of virus spike added (usually expressed as a spike percentage) with throughput capacity. Even with virus spikes of improved purity, impurities in the spike preparation and interactions between the virus spike and protein may limit throughput. Virus spike volumes above 1% of the feed volume should be avoided as these generally impact throughput on Viresolve® Pro Device. Typically, a 0.5% spike is a good starting point. If the feed is known to interact with virus spikes or the baseline and previous process development data show high flow decay (>60%) the spike percentage can be dropped to 0.1% or lower. The balance between spike level and target LRVs should be discussed with personnel in the contract testing laboratory.

Summary Guide of Best Practices

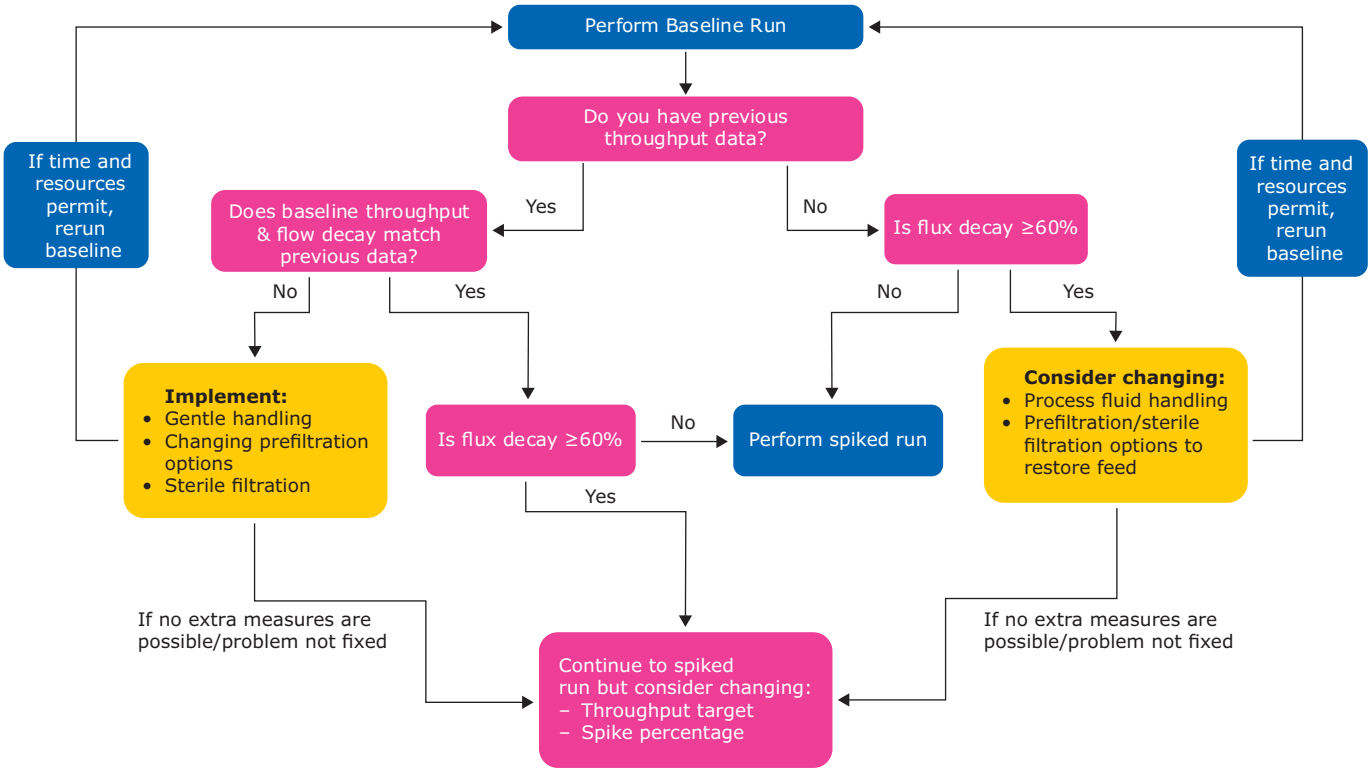

Figure 9. Best practices for running a baseline before spiking studies

References

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