Safety Planning for Virus Volumes Between 100 mL and 10 L

Institutional Biosafety Committee (IBC) — Supplementary Guidance

Approved September 14, 2026

There is no fixed volume that automatically requires BSL-2+ containment.

Neither the *NIH Guidelines* nor the BMBL set a numeric cutoff below 10 liters. Volume alone is a poor predictor of risk because a low-titer, high-volume culture can be safer to handle than a small volume at high titer. Working with more than 100 mL of virus does not by itself trigger enhanced containment; instead, the IBC evaluates each protocol case-by-case based on the information below.

What to Include in the Protocol / Amendment

- Maximum volume ever open/handled at one time — not just the total volume produced or stored
- Vessel/container type and volume in each (e.g.T150 with 25mLs, 1L flask containing 250mL) and whether the procedure is open (e.g., pipetting, pouring) or closed (e.g., sealed bioreactor, capped centrifuge bottle in a sealed rotor)
- Anticipated maximum viral titer in infectious units
- Agent hazard profile: pathogenicity, replication competence, host range, and any genetic modifications
- Aerosol- or splash-generating steps (e.g., vortexing, sonication, centrifugation, syringe use) and the engineering controls used to contain them (BSC class, sealed rotors)
- Written spill containment and cleanup plans specific to the volume and agent, including disinfectant, contact time, and PPE
- Exposure risk to personnel outside of the laboratory (e.g., custodial/EVS staff, individuals who are pregnant or immunocompromised) and how routes of exposure are controlled

Core Safety Practices (scale accordingly as volume increases)

- Perform all open manipulations of virus-containing fluid in a certified biosafety cabinet appropriate to the BSL of the agent
- Use closed or sealed systems (capped bottles, sealed centrifuge rotors/safety cups, closed tubing/bioreactor lines) wherever the procedure allows, especially as volume increases toward the liter scale
- Minimize aerosol generation; no open centrifugation or uncapped vortexing of infectious material
- Have a rehearsed, written spill response plan on hand before starting work, matched to the volume and location of work (bench vs. BSC vs. floor spill)
- Label and post the universal biohazard sign on containers, equipment, and freezers used for the material
- Maintain a written record of the maximum volume and titer actually used, for IBC reporting and periodic review

How the IBC Uses This Information

Reviewers weigh agent hazard, total biological burden (volume × titer), procedure type, and the operational controls described in the application together — not volume in isolation — to determine whether BSL-2 or enhanced BSL-2+ practices are appropriate for each specific protocol. Providing complete, concrete answers to issues above allows for a faster, well-informed determination without triggering an automatic escalation based on the volume alone. If the maximum working volume will exceed 10 liters, then additional large-scale physical containment requirements under the *NIH Guidelines Appendix K* apply and investigators should contact the Biosafety Officer before submission.

Questions? Contact the Institutional Biosafety Division Staff at IBC_Support@pitt.edu before submitting or amending a protocol involving large-volume virus work.