

## Biohazards Assessment in Large-Scale Zonal Centrifugation

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A study was conducted to determine the biohazards associated with use of the large-scale zonal centrifuge for purification of moderate risk oncogenic viruses. To safely and conveniently assess the hazard, coliphage T<sub>3</sub> was substituted for the virus in a typical processing procedure performed in a National Cancer Institute contract laboratory. Risk of personnel exposure was found to be minimal during optimal operation but definite potential for virus release from a number of centrifuge components during mechanical malfunction was shown by assay of surface, liquid, and air samples collected during the processing. High concentration of phage was detected in the turbine air exhaust and the seal coolant system when faulty seals were employed. The simulant virus was also found on both the centrifuge chamber interior and rotor surfaces.

In the last two decades the techniques of zonal centrifugation have proven to be highly successful for isolation and purification of discrete microbial units and subunits. In 1969, N. G. Anderson and co-workers (1), at the Oak Ridge National Laboratories, reported development of a large-scale, K series, continuous-sample-flow-with-banding zonal centrifuge system with flow rate capabilities of 10 liters or more per h. Their objective was single step concentration and purification of particulate antigens for vaccine preparation. The rapidly expanding oncogenic virus program of the national cancer research effort has recently adopted the model K-II centrifuge (Electro-Nucleonics, Inc., Fairfield, N.J.) as a practical method for purifying large stocks of virus (5, 6).

The Office of Biohazards and Environmental Control of the National Cancer Institute investigates potential hazards associated with oncogenic virus research procedures and develops safe alternate practices. The model K centrifuges drew attention because the large volumes of virus processed significantly increase the potential for exposures, and the high concentrations achieved enhance the possibilities of infection from accidental exposure. In addition, many of the viruses being processed are classified as moderate risk agents (2) for which the Office of Biohazards and Environmental Control recommends specific containment and environmental control procedures (2).

A preliminary analysis of the mechanical design and operating practices of the large-scale

zonal centrifuge identified potential problem areas that might create hazardous contamination conditions. The face seals where the hollow rotor shafts interface with the stationary upper and lower continuations of the process fluid channels seemed to possess the greatest potential for release of virus. Leakage could contaminate the face seal coolant system. A poorly operative lip seal could release the contaminated coolant to the turbine air exhaust channels. A catcher-slinger component at the upper end of the rotor system is provided for capture and drainage of the escaping coolant. Interrogation of operators confirmed that lip seal leakage does occur on occasion, as evidenced by excessive drainage from the catcher-slinger port and decreasing volume in the coolant reservoir. Coolant escaping past the lip seal at the lower end of the centrifuge could contaminate the lower damper bearing and its own coolant system. The greatest potential for leakage within the rotor chamber, besides a catastrophic rotor explosion, is at the O-ring seal between the rotor end caps and the rotor barrel (not shown in Fig. 1). Leakage at this point would contaminate the chamber, rotor exterior, and possibly the vacuum system.

Among the personnel procedures observed during the preliminary study that indicated potential hazard from virus aerosol was the occasional removal of the entire upper seal coolant assembly for examination of the face seal while the rotor was spinning. Additionally, collection and manipulation of the gradients

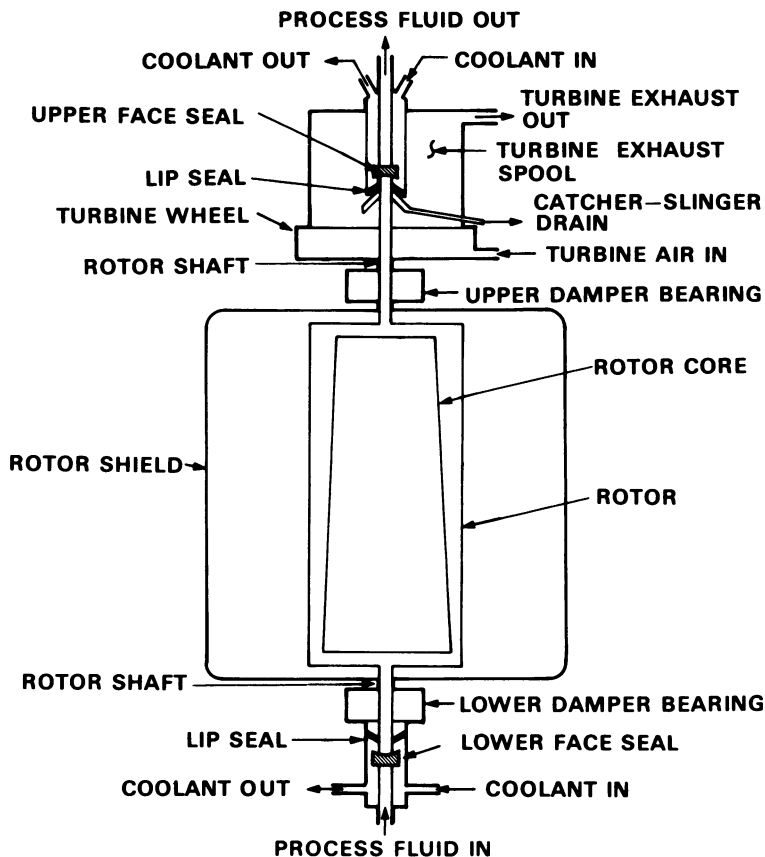


FIG. 1. Diagrammatic sketch of model K-II centrifuge with K3 rotor.

from the rotor after centrifugation appeared to present opportunity for operator exposure to high concentration of virus.

Investigation of laboratory accidents by others indicates infectious aerosols have been associated with the preparative centrifuge (8), but there is no information available on biohazards related to large-scale zonal centrifuges. Our own observations suggested a need for defining safe operating parameters for the K centrifuge system; however, actual measurement of the biohazard involved appeared necessary. A biohazard evaluation study was initiated in a virus production facility (Electro-Nucleonics Laboratories, Inc., Bethesda, Md.) that was providing oncogenic virus to the Virus Cancer Program of the National Cancer Institute. That study, reported here, consisted essentially of substituting a bacterial virus for an oncogenic virus and monitoring the equipment and environment while processing the simulant virus suspension according to oncogenic virus purification protocols.

(This investigation was reported at the An-

nual Meeting of the American Society for Microbiology, Chicago, Illinois, 12-17 May, 1974.)

#### MATERIALS AND METHODS

**Test agent.** The bacterial virus chosen was coliphage T<sub>2</sub> (ATCC 11303-B<sub>2</sub>). The *Escherichia coli* strain used to propagate stock suspensions and assay for phage in the environmental samples was ATCC 11303. All culture and assay work was done in nutrient broth or agar to which 0.5% sodium chloride (wt/vol) was added. Coliphage stocks were prepared by inoculating 4 to 5-h *E. coli* broth culture tubes with reconstituted lyophilized coliphage and incubating at 37 C until lysis was complete. The lysates were clarified by centrifugation, assayed, and stored at 4 C. Lysates assaying at 10<sup>9</sup> to 10<sup>10</sup> plaque-forming units (PFU) per ml were used to inoculate flasks containing 50 ml of 4 to 5-h *E. coli* culture. After incubation the lysates were clarified by centrifugation and filtration through 0.45- $\mu$ m membrane filters. A pooled purified lysate of 10<sup>10</sup> PFU per ml was stored at 4 C and used as the stock for all experimental suspensions. The centrifuge feed dilutions from this stock ranged from 10<sup>7</sup> to 10<sup>9</sup> PFU per ml.

**Sampling.** Air samples were collected by two methods: slit samplers and all-glass liquid impinger

samplers. Slit samplers were of two types: the Fort Detrick slit sampler and the Reynier slit sampler (4, 9), a commercial version of the Fort Detrick sampler; sampling rate of each was verified at 1 ft<sup>3</sup>/min (0.028 m<sup>3</sup>). Collecting medium in the slit samplers was a base layer of agar in a 150-mm petri dish overlaid before use with 20 ml of semisolid agar (0.7% agar-agar) containing 3% (vol/vol) of 18 to 20-h *E. coli* broth culture. PFU were counted directly on the collection plates after overnight incubation at room temperature.

The all-glass impingers were the type with the stem 30 mm from the bottom of the sample tube (9). Collecting fluid in the tube was 20 ml of broth containing 0.06% (vol/vol) Antifoam A (Dow Corning Corp.). Sampling rate was 12.5 liters per min.

Experimental liquid samples, including the all-glass impinger (AGI-30) samples, were assayed for coliphage by plating log dilutions of sample in 10 ml of semisolid agar inoculated with *E. coli* as noted above. The semisolid agar overlaid a sterile agar base in 100-mm petri dishes. Plaques were counted after incubation for 16 h at room temperature and reported as number of PFU per milliliter of undiluted sample.

Surface samples were taken by swabbing 100 cm<sup>2</sup> areas with moistened swabs which were then shaken in 10 ml of broth. The elution broth was assayed as a liquid sample by dilution plating.

Recoverability of coliphage from bearing lube oil, vacuum pump oil, and propylene glycol seal coolant solution was verified by mixing them 1:1 (vol/vol) with a coliphage suspension and quantitating by the above method after a reasonable exposure period. Recovery in each case approximated 100%.

**Experimental runs.** An example of the model K-II centrifuge (with a K-3 rotor) used in the experiments is shown in Fig. 2. It was located in a room having a volume of 870 ft<sup>3</sup> (24.36 m<sup>3</sup>). The room was at negative pressure relative to the adjacent laboratory and had a normal air change rate of 19 volumes per h. After the first run the change rate was lowered to less than 25% to enhance coliphage aerosol collection. Seven runs approximating the processing of oncogenic virus were made. Three of the runs were made under optimal conditions; four runs were performed utilizing damaged face and lip seals.

In each run, the stock coliphage suspension of 10<sup>10</sup> PFU per ml was diluted in 6 liters of broth to give final concentrations of 10<sup>7</sup> to 10<sup>9</sup> per ml. The stationary rotor was layered with 20, 50, and 60% sucrose gradients and slowly accelerated; at 35,000 rpm the flow of phage suspension was started at a rate of 6 liters per h. After the phage suspension had been fed through, the rotor was allowed to run for up to 1 h then slowly decelerated and allowed to stop. Fractions of the gradient containing banded coliphage were collected from the rotor bottom in 100-ml aliquots. These fractions were collected, as normally, in a laminar flow biological safety cabinet; the air flow through the cabinet was turned off to facilitate capture of aerosols by the slit sampler.

During the centrifugation, gradient collection, and centrifuge disassembly, air samples were taken with the Reynier or Fort Detrick slit samplers. The sam-

plers were placed at the room exhaust air grill, atop the centrifuge control console, atop the centrifuge at the slinger drain, at the gradient collection point, and attached directly to the exhaust port of the centrifuge vacuum pump (Fig. 3). An additional sampler was located on the sink in the adjacent laboratory where the rotors were opened. The vacuum pump sampling line was equipped with a relief vent having a bacteriological filter through which particle-free air could be drawn when the vacuum exhaust volume was less than the sampler demand. Air samples were also taken with an AGI-30 sampler tube mounted inside the turbine air exhaust pipe. The sampler tube was pointed upstream of the exhaust flow which had a rated velocity approximately twice that of the sampler intake. Room temperature was maintained at approximately 24 C and relative humidity was elevated above 85% to minimize die-off of airborne coliphage.

Conventional surface swab sampling technique was used to sample the centrifuge chamber interior wall and rotor exterior. The catcher-slinger drain fluid, coliphage flow suspensions, seal coolant, bearing lubricant oil, and vacuum pump oil were assayed by serial dilution plating for quantitation of PFU on *E. coli* lawn plates.

Centrifuge operation was by the same personnel who ordinarily operate the centrifuge for oncogenic virus isolation. Loading gradient and sample, run speed and time, sample collection, and handling were maintained as near as possible to normal oncogenic virus separation procedures.

## RESULTS

With one exception, none of the samples taken during operation of the centrifuge under optimal operating conditions yielded coliphage. Data (Table 1) from the first three runs with damaged seals indicates presence of coliphage in the turbine exhaust and the catcher-slinger fluid drainage. Coliphage was detected on two occasions each on the chamber interior and the rotor surface and on one occasion each in the bearing lubricating oil and the seal coolant fluid. The vacuum pump oil showed no coliphage presence at any time.

As reported in Table 2, release of airborne coliphage into the room appeared to be low; release was most apparent at the slinger drain opening on the top of the centrifuge. During centrifuge operation it is possible to remove the top seal assembly for examination or repair while the rotor is in motion. This operator action was simulated in a fourth malfunction run and the resultant release of coliphage is also recorded in Table 2. A drip pan placed under the centrifuge to collect liquids from the chamber and rotor during removal of the rotor after the fourth run contained 200 PFU per ml. However, the drip pan fluid was also positive for

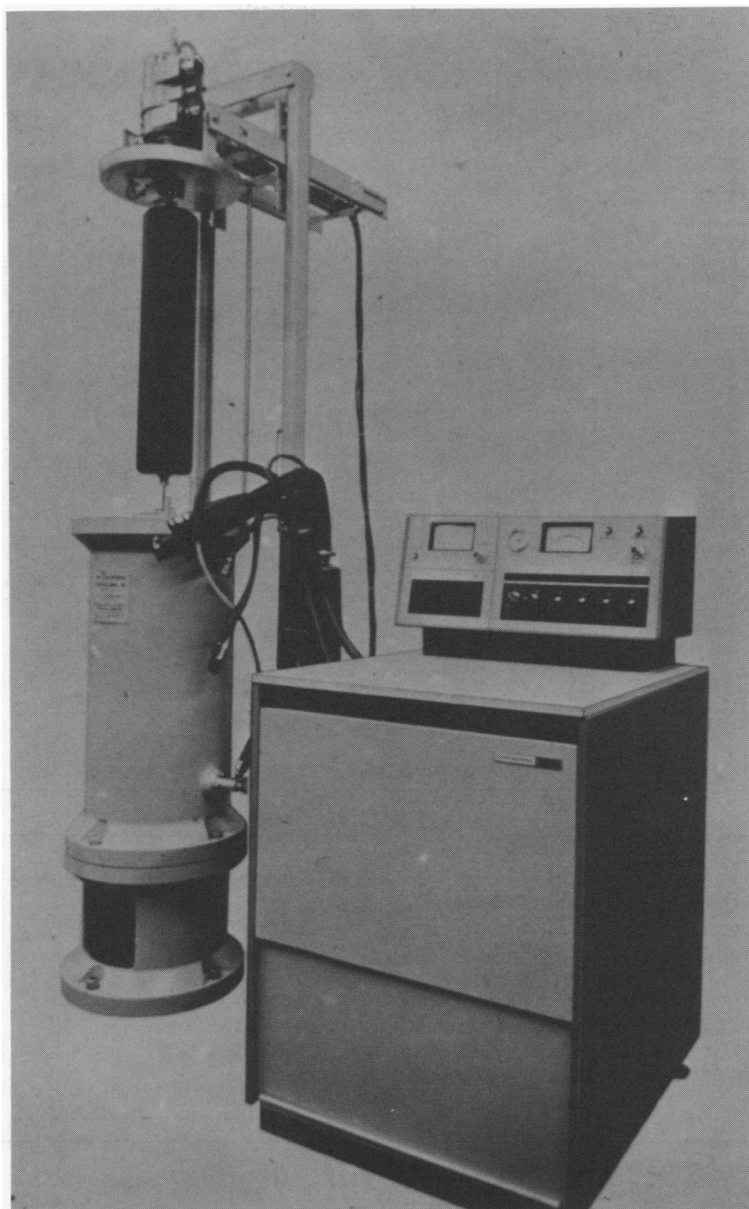


FIG. 2. Model K-II zonal centrifuge and control console. Rotor is in raised position above chamber. (Photo courtesy of Electro-Nucleonics, Inc., Fairfield, N.J.)

coliphage when it was sampled after a run with good seals. Simulant release in this instance probably occurred during disassembly and not during operation of the centrifuge since coliphage was never recovered from the chamber or rotor surfaces after normal runs.

#### DISCUSSION

The sampling data generally appeared to corroborate the initial estimates of virus leakage

patterns. The surface and liquid sample results were as expected but the recovery of aerosolized coliphage in the room was less than anticipated. Only when the top seal assembly was completely removed from the spinning rotor shaft (run No. 4) did the air sampler at the room exhaust grill detect airborne coliphage. It cannot be stated with certainty that there was a complete absence of airborne virus during other malfunction runs; only that factors such as air

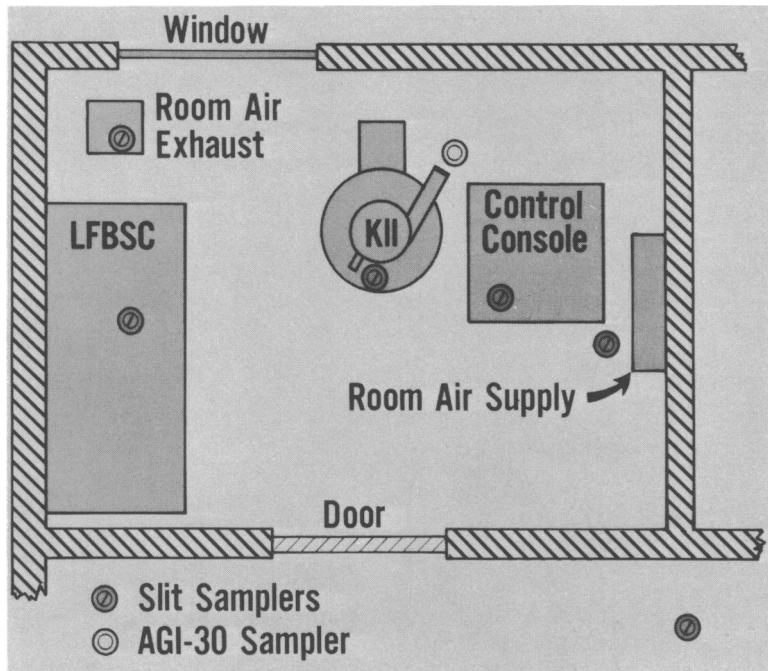


FIG. 3. Air sampler distribution.

TABLE 1.  $T_2$  PFU in  $\bar{K}$ -II centrifuge samples from leaking seal configuration

Sample source	Experimental run		
	No. 1	No. 2	No. 3
Influent reservoir	$10^6/\text{ml}$	$2 \times 10^7/\text{ml}$	$1.5 \times 10^7/\text{ml}$
Turbine exhaust	$2.5 \times 10^6/\text{ft}^3$ (2) <sup>a</sup>	$1.4 \times 10^6/\text{ft}^3$ (6)	$2.8 \times 10^6/\text{ft}^3$ (5)
Chamber wall	$70/100 \text{ cm}^2$ (2)	$120/100 \text{ cm}^2$ (2)	0
Rotor surface			
Top	$80 \times 10^6/100 \text{ cm}^2$	$2 \times 10^6/100 \text{ cm}^2$	0
Bottom	0	$3 \times 10^6/100 \text{ cm}^2$	0
Catcher-slinger	$30.5 \times 10^6/\text{ml}$ (4)	$4.5 \times 10^6/\text{ml}$ (2)	$8.8 \times 10^6/\text{ml}$ (3)
Bearing lube oil	$2 \times 10^6/\text{ml}$	0	0
Seal coolant	ND <sup>b</sup>	$4 \times 10^6/\text{ml}$	0
Vacuum pump oil	0	0	0

<sup>a</sup> Parentheses show number of samples assayed to obtain average.

<sup>b</sup> ND, Not done.

dilution and die-off may have kept the levels below the sensitivity of our detection system. Neither can it be stated that there was no virus aerosol release into the room during the three optimal runs; however, absence of positive liquid and surface samples enhance credibility of these negative air samples.

A study by Warren and Hatch (7) reported relative humidity in excess of 80% was conducive to highest recovery rates of  $T_2$  coliphage from aerosols. Room humidity was artificially elevated above 85% in this investigation for

maximum air sampling efficiency. High humidity may or may not be beneficial to survival of airborne oncogenic viruses, but enhancement of simulant virus survival in the present experimental model is essential.

It has been concluded from the room air sample data that during optimal centrifuge operation the hazard to the immediate environment is minimal and that primary containment for the centrifuge is not justified when processing low to moderate risk oncogenic agents. However, since aerosolization is possible

TABLE 2.  $T_1$  PFU detected in air samples during K-II centrifugation with leaking seals

Sampler location	Experimental Run			
	No. 1	No. 2	No. 3	No. 4
Slit Sampler				
Exhaust grill	0	0	0	6.4/ft <sup>3</sup> <sup>a</sup>
Centrifuge console	0	ND <sup>b</sup>	ND	ND
Top of centrifuge	TNTC <sup>c</sup>	0.46/ft <sup>3</sup>	0	2.6/ft <sup>3</sup> <sup>a</sup>
Vacuum pump exhaust	ND	0	0	1.0/ft <sup>3</sup> <sup>a</sup>
Rotor opening	1.4/ft <sup>3</sup>	0	0	ND
Bottom of centrifuge	ND	ND	ND	1.8/ft <sup>3</sup> <sup>a</sup>
Drip pan (liquid)	ND	ND	ND	2 × 10 <sup>3</sup> /ml <sup>d</sup>

<sup>a</sup> Period immediately after temporary removal of top seal. Cubic foot is equal to 0.028 m<sup>3</sup>.

<sup>b</sup> ND, Not done.

<sup>c</sup> TNTC, Too numerous to count (but only while slinger drain was open).

<sup>d</sup> A sample from a normal run with good seals gave 2.6 × 10<sup>3</sup>/ml.

through accidental feed line rupture, spills, or removal of the top seal assembly during rotor operation, isolation of the centrifuge in a separate room is recommended. The National Cancer Institute laboratory design criteria for basic research facilities (3) recommends that the room be at negative pressure relative to adjacent areas, have an air change rate not less than 10 per h, and be supplied with 100% fresh outdoor air, and the exhaust should not recirculate through the ventilation system. The National Cancer Institute safety standards (2) require that if recirculation of the room air is necessary it should first be appropriately treated, e.g., removal of airborne particulates by filtration.

The presence of the coliphage in turbine effluents, both liquid and air, during the simulated malfunction likewise confirms early estimates of leakage potentials. Calculations based on the coliphage levels quantitated in the turbine air exhaust (approximately 2,900 feet [ca. 883.8 m] per min) indicate potential for as much as  $1.2 \times 10^8$  viable particles per min or  $2.3 \times 10^6$  per ft<sup>3</sup>. Since the all-glass impinger sampling velocity (approximately 1,400 feet [ca. 426.8 m] per min) was only about one-half that of the turbine exhaust stream the actual values may be considerably greater. This concentration of moderate risk agent is sufficient to warrant high efficiency particulate air filtration of turbine effluent before release to the external environment. The filters must be protected by condenser traps that remove oil mist from the exhaust air flow.

Filtration of the turbine exhaust is even more justified for the later model turbines which purge all lip seal leakage via the exhaust air flow rather than capture and drain it to the outside

through a catcher-slinger component. The experimental coliphage data shows that if an externally draining catcher-slinger configuration is employed, good safety practice dictates that both air and fluid emissions at that point should be trapped in a disinfectant-containing receiver having a vent fitted with a bacteriological filter. Some operators prefer an open catcher-slinger drain because emissions at this point indicate operating performance of the lip seal.

The demonstration of simulant virus in the seal coolant system is consistent with malfunctioning face seals. It also points out the advisability of decontamination of that entire system before disassembly. Decontamination may be achieved by addition of iodophor to the coolant reservoir and continuing circulation during similar decontamination of all virus influent and effluent lines of the rotor.

Coliphage found on the chamber walls and on the rotor surfaces may have come from incomplete sealing of the rotor end caps or from migration down the rotor shaft from the lip seal area. When asked for a most likely explanation a consensus of experienced operators (C. L. Baldwin, ed. 1974. Proc. Symp. Centrifuge Biohazards, in press) indicated the contamination probably arose through a faulty end cap seal. Regardless of origin, the demonstrated potential for contamination of these areas suggests the need for effective surface decontamination to protect the operators at time of rotor removal. Good practice further dictates total immersion of rotor, end caps, and core in disinfectant solution before cleaning.

The presence of coliphage in only one sampling of the vacuum pump exhaust is inconclusive. The phage was detected during the time

the top seal was removed from the rotor and may actually represent a sampling of room air through a leaky filter on the vacuum pump sampler relief system. Passage of viable virus through a vacuum pump has been observed. Braymen and Songer, in the cited symposium on centrifuge biohazards, reported as much as 3.2% of coliphage S<sub>13</sub> challenge aerosol passing through an operating ultracentrifuge vacuum pump under use conditions.

The results of these biohazards assessment experiments suggest application of several contamination control procedures and facility design features in the interest of personnel safety. The safe operating practices for processing low and moderate risk oncogenic viruses in the model K-II centrifuge should include: (i) isolation of the instrument in a room having negative relative pressure and no air recirculation unless treated by high efficiency particulate air filtration; (ii) high efficiency particulate air filtration of the turbine exhaust air; (iii) entrapment of catcher-slinger drain discharges; (iv) postoperation decontamination of coolant systems and all feed and effluent lines; (v) surface decontamination of rotor and wall of chamber; (vi) respiratory protection, gloves and lab coat on operators when seal is removed during rotor operation; (vii) gloves and protective garments for operators when removing and cleaning rotor; and (viii) collection of gradient fractions and other manipulations of concentrated virus confined to a safety cabinet.

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