2. Injections should be carried out once a week for 4 wk, and a test bleed should be taken 14 d after the last immunization.
3. When the desired titer of specific antibody has been achieved, collection of blood can begin. Regulations concerning maximum volumes of blood that can be collected vary among countries and the appropriate authorities should be consulted.
4. If large volumes of serum are required, then animals can be given boost injections after 6 wk and bleeding can recommence after 7–10 d.
5. Blood should be allowed to clot at 4°C overnight and the serum should be collected by slow centrifugation.

3.2. MAb Production

3.2.1. Immunization Protocol

1. Mix 50–100 μg of purified virus preparation (*see* **Notes 7** and **8**) for each mouse, with an appropriate adjuvant to a final volume of 100 μL in PBS. This is injected intraperitoneally, or subcutaneously into the neck scruff.
2. Injections should be carried out on d 0, 14, 28, and 56. Immune status should be checked by a test bleed 10 d after the last injection. Blood can be obtained by performing tail-tip amputation under anesthesia with ether.
3. Seventy-two hours before the cell fusion is performed, a final boost of 50–100 μg of virus should be given without adjuvant. The injection site should be either intraperitoneal or intravenous. If the intravenous route is to be used, then the amount of virus given should be reduced to approx 20 μg.

3.2.2. Myeloma Cell Preparation

Several myeloma cell lines have been developed for monoclonal antibody production. The line must be hypoxanthine guanine phosphoribosyl transferase (HGPRT) defective, so that it is sensitive to aminopterin, and must not secrete its own antibody (*see* **Note 6**). The enzyme deficiency is required so that only recombinant cells can grow in the HAT medium as they inherit the necessary enzyme pathway from the parent spleen cell. Cell lines derived from the NS1 myeloma, such as NS 0 *(7)*, are ideal for MAb production.

Culture the cells in a 37°C incubator with 5% CO_2 and 90% humidity for several days prior to cell fusion, at subconfluent numbers to ensure log growth. Myeloma cells are fairly undemanding and can be grown in RPMI-1640 medium containing only 5% FBS.

3.2.3. Preparation of Spleen and Myeloma Cells for Fusion

The immunized mouse is killed by cervical dislocation and the spleen removed aseptically. The spleen is placed in a sterile 25-mL universal container containing sterile PBS and is kept at 4°C. The splenocytes are obtained by homogenizing the spleen either with a manual tissue homogenizer or by grinding between two frosted-glass slides. Suspend the cells in PBS and wash