

Studies on the Clearance of DNA and virus from Protein solution by Membrane

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Abstract

In this study used membrane filters, this is one of the physical methods, to conduct research about the analysis of the removal of DNA and virus as two of the sources of biomedical product contamination. First, we used membrane filters to eliminate DNA from solution. The membrane filters used for DNA elimination were .2um membrane filter and anion exchange membrane filter (Q15x). After measuring the efficiency by using each filter, we found excellent result that Q15x using change had elimination efficiency of at least 4.5 log, the chromatography was reusable, and it had advantage that the disinfection can be done at the temperature of 120°C. Second, we used membrane filters to eliminate virus from protein solution. The virus used was HCMV, and MRC-5 cell was used for the cultivation of virus. Then, we used .2um membrane filter, Q15x, cat-ion exchange membrane filter (S15x), and nano filter in order to eliminate the virus. After we assessed the efficiency by measuring the number of virus derived from each filter, it was found the result of >4.31, >4.19, >4.62, >4.71, and among the filters, nano filter showed excellent elimination rate; however, after we compared the purity measured the amount of protein which was collected at the last stage, it was found that S15x which uses unique structure of virus showed high elimination rate as high as 98.6%.

Key Words ; HCMV, MRC-5, S15x, Q15x, nano filter

References

1. Kohler, G. and Milstenin, C. (1975), *Nature* **259**, 495.
2. Peter, R. (1997), Efficient removal of viruses by a novel polyvinylidene fluoride membrane filter, *J. Virological Methods* **65**, 27.
3. Nigel, K. H., Slater, H., and Cherlton, R. (1999), Characterisation of a generic

- monoclonal antibody harvesting system for adsorption of DNA by depth filters and various membranes, *Bioseparation* **8**, 281.
4. Griffiths E. (1997), WHO expert committee on biological standardization highlights of the meeting of October 1996, *Biologicals* **25**, 359.
 5. WHO (1997), Acceptability of Cell substrates for Production of *Biologicals*, 747.
 6. ICH (1998), Guidance on Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin; Availability.
 7. Atkinson A and Jack G. W. (1973), Precipitation of nucleic acids with polyethyleneimine and the chromatography of nucleic acids and proteins on immobilised polyethylencimine, *Biochimica and Biophysica Acta* **308**, 41.
 8. In Mandell G. L, Douglas R. G. Jr. and Bennett J. E (1990), Principles and practice of infectious diseases. 3rd ed. Churchill Livingstone Inc., New York.
 9. In Joklik W. K., Willett H. P., Amos D. B., and Wilfert C. M. (1988), *Zinsser Microbiology*, 19th ed. Appleton & Lange, East Norwalk.
 10. In Becker Y., Darai G., Huang E. S. (1993), Molecular aspects of human cytomegalovirus diseases. 1st ed. Springer Verlag, Berlin.
 11. Stiehm E. R. (1997), Human intravenous immunoglobulin in primary and secondary antibody deficiencies, 16.
 12. Dwyer J. M. (1996), Immunoglobulins in autoimmunity : history and mechanism of action, 15.
 13. Ramesh S, Schwartz S. A. (1995), Therapeutic uses of intravenous immunoglobulin (IVIg) in children, 16.
 14. Noreen M. Troccoli (1998), Removal of Viruses from Human Intravenous Immune Globuline by 35nm Nanofiltration, *Biologicals* **26**.
 15. Jun Tateishi (2001), Scrapie Removal using Planova^(R) Virus Removal Filters, *Biologicals* **29**.
 16. Tateishi J., Kitamoto T. (1993), Removal of causative agent of Creutzfeldt-Jakob disease (CJD) through membrane filtration method.
 17. Tateishi J., Kitamoto T. (1995), Removal of the prion protein using validatable filter. In: Beuvery EC *et al.* Animal cell technology: Development towards the 21st century. Netherlands, Kluwer Academic Publishers.
 18. Yan Jin, Yanjie Chu (2000), Virus removal and transport in saturated and unsaturated

- sand columns, *Contaminant Hydrology* **43**, 111.
19. Klaus Hamprecht (1998), Matthias Vochem , Detection of cytomegaloviral DNA in human milk cells and cell free milk whey by nested PCR, *J. Virological Methods* **70**, 167.
 20. L. Barber, J. J. Egan (1999), The development of a quantitative PCR ELISA to determine HCMV DNAemia levels in heart, heart:lung and lung transplant recipients, *Virological Methods* **82**, 85.
 21. Barber, L., Egan, J. J. (1996), Comparative study of three PCR assays with antigenaemia and serology for the diagnosis of HCMV infection in thoracic transplant recipients, 49, 137.
 22. Van der Bij, W. (1998), Torensma, R. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leukocytes, *J. Med. Virol.* **25**, 179.
 23. Committee for Proprietary Medical Products (CPMP) Ad Hoc Working Party on Biotechnology/Pharmacy. Note for guidance: validation of virus removal and inactivation procedures. (1991) 89.
 24. Yamamoto Y., Haroda S. (1994), A novel method for removal of human immunodeficiency virus: filtration with porous polymeric membranes, 56: 230.
 25. Shengjiang Liu, DeJin Zhan (2002), Detection of Minute Virus of Mice Using Real Time Quantitative PCR in Assessment of Virus Clearance During the Purification Of Mammalian Cell Substrate Derived Biotherapeutics, *Biologicals* **30**, 259.
 26. Lena Miloradovic, Ian Cheyne and Kevin Healy (2000), The Removal of Viruses During the Purification of Equine Antisera using Filtration Aids Hyflo Super-CelY and FulmontY Super A Rachel Cameron-Smith, *Biologicals* **28**, 169.
 27. Yuan Xu, Allen S. L., Lau, Yolanda S. Lie 1 (1999), Quantitative competitive reverse transcription-PCR as a method to evaluate retrovirus removal during chromatography procedures, *J. Biotechnology* **75**, 105