Methods 134–135 (2018) 87–97

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Flow virometry as a tool to study viruses

J. Lizbeth Reyes Zamora, Hector C. Aguilar *

Department of Microbiology and Immunology, Cornell University, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 15 July 2017
Received in revised form 10 December 2017
Accepted 14 December 2017
Available online 16 December 2017

Keywords:
Flow virometry
Flow cytometry
Sorting
Virus
Glycoprotein
Vaccine

ABSTRACT

In the last few decades, flow cytometry has redefined the field of biology, exponentially enhancing our understanding of cells, immunology, and microbiology. Flow cytometry recently gave birth to flow virometry, a new way to detect, analyze, and characterize single viral particles. Detection of viruses by flow cytometry is possible due to improvements in current flow cytometers, calibration, and tuning methods. We summarize the recent birth and novel uses of flow virometry and the progressive evolution of this tool to advance the field of virology. We also discuss the various flow virometry methods used to identify and analyze viruses. We briefly summarize other applications of flow virometry, including: virus detection, quantification, population discrimination, and viral particles' antigenic properties. Finally, we summarize how viral sorting will allow further progress of flow virometry to relate viral surface characteristics to infectivity properties.

Contents

1. Introduction: History of flow virometry . . . 88
   1.1. Early virus detection . . . 88
   1.2. Early virus staining . . . 88
   1.3. Viruses captured on scaffolds . . . 88
   1.4. Direct detection of viral particles using antibodies . . . 88
   1.5. Viral sorting . . . 88
2. Flow virometry equipment and methodologies . . . 88
   2.1. Flow cytometer recommendations . . . 88
   2.2. Flow cytometer analysis preparation . . . 90
   2.3. Virus dilution and adjustable settings prior to analysis . . . 91
   2.4. Virus staining methods . . . 92
      2.4.1. DNA and RNA labeling . . . 92
      2.4.2. Scaffold labeling . . . 92
      2.4.3. Direct viral labeling . . . 94
3. Troubleshooting . . . 94
4. Flow virometry applications . . . 94
   4.1. Flow virometry for virus quantification . . . 94
   4.2. Flow virometry for virus surface glycoprotein and lipid composition . . . 95
   4.3. Virus sorting . . . 95
   4.4. Flow virometry for vaccine quality assurance . . . 95
5. Conclusions and future applications . . . 95
   Acknowledgements . . . 96
   References . . . 96

* Corresponding author at: Department of Microbiology and Immunology, Cornell University, 930 Campus Road, Ithaca, NY 14853, USA.
E-mail address: ha363@cornell.edu (H.C. Aguilar).

https://doi.org/10.1016/j.ymeth.2017.12.011
1046-2023/

1. Introduction: History of flow virometry

Traditional use of flow cytometers involves detection of cells, cell populations and antigens on these cells [1]. However recent advances in the field of flow cytometry have allowed the detection of microparticles that range between 100 nm and 1000 nm in size. These microparticles can be components of cells, for example exosomes, or intruders of cells, such as small bacteria or viruses [2–30]. Flow virometry refers to the use of a flow cytometer to detect viral particles. Viruses and/or viral particles are commonly examined via transmission electron microscope (TEM), epifluorescence microscopy (EFM), ELISA, titration assays, western blot analysis, and PCR methods. However, these methods analyze viral preparations in bulk, and often require toilsome sample preparation. Major drawbacks of these techniques include the inability to perform fast-throughput analyses, the inability to produce data for individual virions or their proportions in viral preparations, and the lack of discrimination between virions and non-infectious viral particles. The advent of flow virometry, however, allows for the direct detection of single viral particles as well as their characteristics [4–10, 15–17,19–29]. Like conventional flow cytometry staining procedures, viruses are typically first isolated and incubated with a stain, and then analyzed through a flow cytometer that can detect viral particles. As flow virometric analytic tools have only been recently available, they have slowly evolving applications. This review addresses the development of flow virometry and the various methods used to analyze viruses.

1.1. Early virus detection

Earlier flow cytometer models had major restraints on the size and type of samples they could detect. Detection of viruses became possible when Hercher et al. designed a custom flow cytometer in 1979 [4]. This cytometer was built to stream viruses through a sheath-fluid-containing capillary and central core by a microliter pump. The diameter of the stream was 200 μm, and the core diameter 2–20 μm. This decrease in cross-sectional area pushes particles through a smaller volume, highly encouraging single-particle flow. Moreover, lasers were focused and magnified through the observation plane of the capillary using microscope magnifiers. Using this custom-built flow cytometer, Hercher et al. were able to detect T2 bacteriophages (60 nm head and 120 nm tail) distinctly from background noise or from reovirus (60–80 nm) populations based on light scattering profiles [4]. Although viral detection by light scattering properties was possible, not much information could be determined based on light scattering profiles. The development of a flow cytometer capable of detecting viruses, however, was a first step in opening a new field of research.

1.2. Early virus staining

The development of flow cytometers with microcapillary fluidic systems and stronger lasers along with the discovery of stable and superior DNA staining dyes allowed for detection of fluorescently-labeled viruses. In 1999, Marie et al. labeled marine bacteriophages using a novel SYBR Green-I nucleic acid stain [5]. Detection of viruses by flow cytometric means was further expanded to viruses from different families, for example, *Baculoviridae*, *Herpesviridae*, *Myoviridae*, *Phycodnaviridae*, *Picornaviridae*, *Podoviridae*, *Retroviridae* and *Siphoviridae* [6]. With the development of more stable and strongly fluorescent nucleic acid stains, such as SYBR-Gold, marine viruses were better stained and detected [7]. Throughout the aforementioned studies, viruses were visualized by genome staining. However, only DNA viruses (larger viruses) were successfully detected.

1.3. Viruses captured on scaffolds

Further progression of flow virometry involved capturing viruses on scaffolds. Scaffolds in an earlier study involved the use of microspheres coupled with antibodies and a later group used discretely sized magnetic nanoparticles. Microsphere/nanoparticle-bound viruses were then stained by targeting antigens on viral particle surfaces with fluorophore-conjugated antibodies. Yan et al. used microspheres coupled to antibodies to capture and differentiate between Influenza A and Influenza B viruses. Another group used a magnetic-nanoparticle type of scaffold to capture HIV-1 and Dengue viruses [16,22,27]. These studies opened up the field of flow virometry to the detection of viral glycoproteins on the viral surface.

1.4. Direct detection of viral particles using antibodies

Direct detection of viral like particles (VLPs) either alone or by the use of antibodies was initially achieved by Landowski et al. Nipah VLPs were purified and stained with primary and secondary antibodies and detected via a flow cytometer capable of small particle detection [17]. This was the first time viral particles were stained with antibodies to detect viral glycoproteins without the use of scaffolds. Importantly, these virions were detectable solely by forward and side scatter parameters, without the need of fluorescent labels, allowing for quantification of total numbers of virions. The following year Gaudin et al. used a similar flow virometric approach to detect Junin virus using a non-neutralizing antibody that recognizes the Junin virus glycoprotein [21].

1.5. Viral sorting

The Allen, Khalil, and Gaudin groups took it a step further and sorted bacteriophages, marine amoebic viruses, and Junin viruses, respectively. A mixture of λ and T4 *E. coli* bacteriophages were sorted for further downstream genomic characterization [19]. Mimivirus and Cedratvirus were successfully sorted from amoebic co-culturing supernatants [29]. Junin virus was sorted onto grids to recapitulate size of particles via EM analysis as well as develop an infectivity profile that reflected virus size, viral surface glycoprotein, and lipid raft content [21]. Likewise, Bonar et al. applied flow virometry to HIV-1 detection and further sorting, and importantly, the sorted HIV-1 retained infectivity [25]. Furthermore, Bilali et al. reported a correlation between high levels of HSV-1 tegument proteins (VP16 and VP22) and infectivity [26].

2. Flow virometry equipment and methodologies

Flow virometric analysis requires careful consideration at many steps, including: the type of instrument used, background determination, calibration method, virus preparation and staining. Depending on the question at hand, an investigator may opt to use a particular flow cytometer and a particular method. These next sections aim to outline various flow virometric techniques and recommendations.

2.1. Flow cytometer recommendations

Detection of viruses via flow cytometry was possible after customized improvements on flow cytometers [4,8]. These improvements eventually led to the creation of modern flow cytometers that have a lower detection limit for smaller particles. It is important to note that the majority of flow cytometers are not built for the purpose of detecting smaller particles such as viruses, however, modifications to a flow cytometer in combination with background