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From the Front Lines of Infection Prevention: Using Electron Microscopy in a Public Health Research Center

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1. Introduction

Japan has Prefectural and Municipal Public Health Institutes (PHIs) in all of the country's 47 prefectures and in designated cities. The institutes act as public organizations that support public health policy in their jurisdictions, serving as bases of scientific and technical expertise. Their four core competencies are survey-based research, experimental research, training/guidance, and the collection and analysis of public health information. In the early stages of the COVID-19 pandemic, the National Institute of Infectious Diseases and PHIs nationwide met the country's PCR testing needs.

The institutes stand ready to be first responders to future emerging and re-emerging infections. Given the important role of the PHIs in risk management, lawmakers amended the national Community Health Act in December 2022 to formalize their establishment in law. In 2017, the institutes of Osaka Prefecture and the city of Osaka were consolidated with the goal of enhancing their ability to implement public health policy, becoming Japan's only Incorporated Administrative Agency responsible for public health. The integration was finalized in January 2023 with the completion of the centralized facility, and an HT7800 transmission electron microscope (TEM) was installed.

2. Virological Investigations and Electron Microscopy

Back in my days in the laboratory in university, I remember hearing that a group of small, round viruses called small round structured viruses (SRSVs) would be officially recognized as a source of foodborne illness. SRSVs include noroviruses, and this marked the beginning of my involvement with them. Since many SRSVs defy culturing, researchers studied them by directly observing virus particles in the fecal samples of patients using electron microscopy (EM). A report written by an epidemiology team responsible for tracing the causes of non-bacterial foodborne illness stated that about half of the PHIs use EM, and that EM was used in about 70% (602 of 908) of the cases of non-bacterial foodborne illness investigated[1]. These figures highlight how important a tool the electron microscope is.

Researchers are able to quickly draw conclusions provided virus particles are imaged by EM. Virus particles that are small or few in number, however, can evade detection. Wanting to improve detectability, we looked at how many particles in viral suspensions were adsorbed to EM grids after different adsorption times. (Grid adsorption is typically done for 3 minutes.) I found that counts peaked at 8 hours, declining thereafter (Figure 1). The findings are based on just a few runs, but they indicate that increasing adsorption time may be helpful when the sample contains few virus particles.

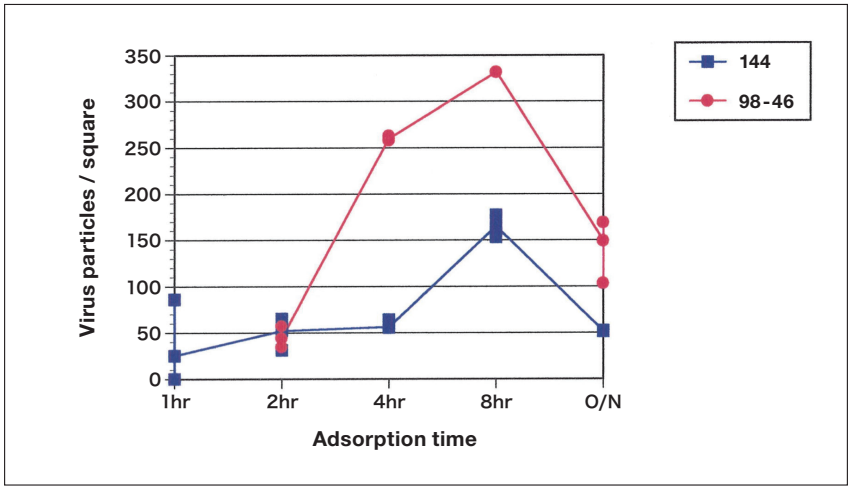


Fig. 1 Investigation of grid adsorption times
The investigation used samples positive for norovirus, which is normally observable with 3 minutes of adsorption.

3. Application of EM Techniques to Risk Management

As rapid gene tests replace EM-based testing, fewer researchers are able to perform the laborious, multi-step process of EM sample preparation, operate electron microscopes, and differentiate virus particles based on morphology. This situation could make it difficult to pass along the techniques involved to future researchers. To address this issue, Toshiyuki Goto (electron microscopist, deceased) of present-day Osaka Medical and Pharmaceutical University encouraged Isao Oishi and his colleagues (of the former Virology Section of the Osaka Institute of Public Health) to join the External Quality Assurance Program for Diagnostic Electron Microscopy of Infectious Diseases (EQA-EM) of the Robert Koch Institute in Germany. Dr. Hans Gelderblom leads this project at the Robert Koch Institute (Figure 2). Three times a year, he would assess the findings we made in EM observations of inactivated virus samples he sent. We still do this once yearly.

Dr. Gelderblom visited Osaka in 2004, giving a presentation at the Osaka Institute of Public Health on February 18. He discussed how useful EM can be in risk management. His presentation covered Mpox (previously known as monkeypox), which is now in the news, and touched on techniques for collecting hazardous samples in the event of a hypothetical smallpox bioterrorism attack[2]. I was surprised when he told us about a safe and rapid technique with which samples collected from the pustules of a patient using a syringe containing an inactivating agent could be viewed directly using EM. The EQA-EM program holds that EM is an important tool in virus diagnosis because of its ability to differentiate blister-forming pathogenic viruses from related diseases, and under the program, I have gained experience observing orthopoxvirus, orfvirus (*Parapoxvirus*), and herpesvirus samples[3]. The program has also let me observe the then newly discovered mimivirus, which does not appear in virus guides at that time. We received a sample of SARS-CoV soon after the 2003 outbreak of SARS in Hong Kong. The keys to proper virus observation are observation and vigilant collection of information on potential outbreaks and the virus responsible.

From left to right, Dr. Goto, Dr. Utagawa, Dr. Gelderblom's wife, and Dr. Gelderblom

Dr. Gelderblom

Fig. 2 Dr. Gelderblom in a photo taken during his visit to Osaka

4. Partnering with the Next Generation of Researchers

When the prefectural and municipal institutes were consolidated into one institute, we procured a Hitachi HT7800 electron microscope. This let us resume EM after a two-year hiatus. This new model featured better operability than the electron microscope we previously used. I was also pleasantly surprised to find that the peripherals were quite easy to use. Operability previously posed a hurdle to those interested in starting EM, but I was convinced that researchers with little experience would be able to use the current instrument we have.

We started by training young researchers about a process spanning from instrument setup to electron microscope operation. Specifically, we prepared samples with virus-inactivating agents and phosphotungstic acid (PTA) stain and made grids with support films and carbon coating. Figure 3 shows a grid made with a formvar support film. Although grids are commercially available, I find grids made in-house to remain more stable during EM observation than purchased grids, because we must place viral suspensions and stains on our grids. Next, we had our trainees culture, inactivate, and concentrate viruses and then observe them. A description of the EM processes and resulting images of virus particles are stored in our library for future use.

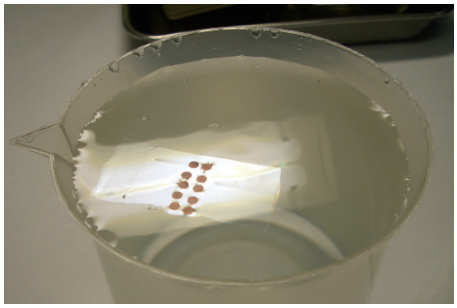
a)

400-mesh copper is deparaffinized

b)

Microscope slides are coated with formvar

c)



Formvar is floated on water and placed on the mesh

Fig. 3 Excerpted from training materials used in training in formvar film preparation
The classical tools remain in use.

We held what we called the “Experience Science over Summer Vacation” at the institute on August 5, 2024 for elementary school children. Since we wanted to show participants the microscopic world, we asked Hitachi High-Tech for help. We quizzed participants in the three categories of optical microscopy, scanning electron microscopy (SEM), and electron microscopy (Figure 4). Samples included eggshells, salt, herbs, and other familiar items, as well as ants and the scales on butterfly wings. The children selected and prepared samples and then viewed them under a scanning electron microscope that they operated. The participants had fun preparing samples and answering quizzes.

Participants answer a quiz

A participant observes a sample they prepared

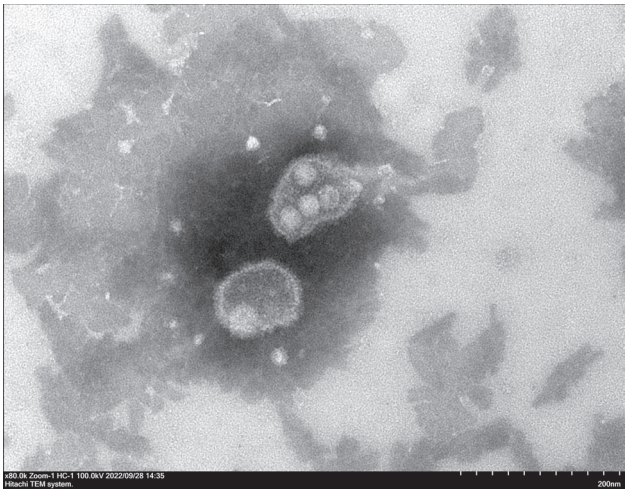
Fig. 4 “Check Out the Microscopic World” in “Experience Science over Summer Vacation”

5. The Future Role of EM at Other Public Health Institutes

The COVID-19 pandemic changed our way of life. It also prompted us to think about how the virus actually exists and its dynamics in the environment. Coronaviruses cause mostly respiratory diseases, but they can grow in the intestines. (A bovine coronavirus is the cause of diarrhea.) Knowing this, experts predicted that SARS-CoV-2, which causes COVID-19, might be detected in sewage. Many studies have found a correlation of new infections to viral concentrations in sewage, accelerating the discipline of wastewater surveillance. Those engaged in virus research (and the observation of virus particles in particular) know how important it is to eliminate impurities when concentrating and purifying samples. What surprised us was the presence of many viruses in the removed impurities as detected with gene analysis. Here at the Osaka Institute of Public Health, we perform wastewater surveillance by identifying viral genes in samples pelleted by centrifuging sewage.

Wastewater surveillance uses techniques different from conventional virus concentration procedures. In addition, new findings have been made in observations of viruses in rotavirus- or norovirus-positive stool[3]. Exosomes recovered from the stool of infected people contain structures called vesicles that hold several virus particles. Researchers postulated that these structures could increase the environmental resistance and infectivity of viruses. We repeated these observations, finding vesicles in the stool of norovirus-positive patients collected through exosome recovery (Figure 5). EM may hold the key to unlocking viral kinetics.

It will be important to trial previously overlooked techniques such as detecting viruses in sediment and observing vesicles.



Norovirus particles (exosomes collected from norovirus-positive stool)

Vesicle in rotavirus A culture medium

Fig. 5 Micrographs of vesicles
Several virus particles are seen in a membrane-like structure.

6. Conclusions

We began using TEM and SEM more in our work after we purchased the new electron microscope. Many public health institutes are nearing the time when they need to replace their electron microscopes, but now that gene testing has become the predominant testing method, fewer PHIs are considering doing so. It would be a good way to share and manage EM among PHIs in the same region. I see a real need for PHIs to build a network for sharing and observing virus-positive samples for EQA-EM purposes so they can maintain their competence in EM techniques. Since multiple researchers can now concurrently observe samples online, the time has come to use new systems to strengthen partnerships. I seek cooperation in training a broad array of personnel in local areas through internal and external training and partnerships so that we can more accurately and quickly engage in public health policy for risk management and use these techniques in our research.

Acknowledgments

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