First, some gossip. During a meeting last year, I heard a story from the director of a middle-ranking medical microbiology laboratory in southern Europe. Staff there had been perplexed by the unexpected, unexplained detection of a coryneform bacterium during routine screening of swabs from a nearby hospital. On five separate occasions, the organism had appeared on MacConkey agar and other cultures, and was at first difficult to identify. Only after the apparent contaminant was sent for further investigation to a reference laboratory elsewhere was part of the mystery solved. There, 16S rRNA gene sequencing showed the organism to be \textit{Brevibacterium otitidis}.

I say “part of the mystery” because definitive identification of the bacterium still left unanswered the question of its origin. Maddeningly, the organism had turned up on plates being used to investigate specimens from five different patients. Could contamination of the culture media or their constituents be to blame? Careful searches proved negative, as did screening of the staff. Was there an unapparent source on the benches or other parts of the lab? Diligent scrutiny again proved negative.

But then the problem suddenly disappeared—and this led to a likely explanation. Someone noted that the cluster of five cases came to an end immediately after a student intern, employed at the center during the summer holidays, went away. Oddly, as a temporary worker, he was the only person around the lab who had not been swabbed. But as he was now many miles away on another campus, he was not investigated. The truth seemed inescapable—though not, of course, proven.

Cases of this sort are probably quite rare. The last one about which I myself heard first-hand evidence concerned an elderly veterinarian in the north of England over 40 years ago. To his acute, indeed chronic, embarrassment, the source of a contaminating virus in the center where he worked proved to be his own pet cat. Even when the cat fell under suspicion and was screened, it took over a week for a clear verdict to emerge. As the contaminant was apparently an enterovirus, the animal’s feces were thoroughly scavenged, but with negative results. Eventually, the problem turned out to have been originated by an ocular infection—which was quite obvious even on casual examination but which the vet had not noticed because he was far more used to dealing with racehorses and farm livestock than with cats and dogs. Ironically, he also had rather poor eyesight. As I said, the incident was all very embarrassing.

Although unusual, such episodes do remind us that the caricature of white-coated sleuths, wholly insulated from the microbial domain they are studying, is indeed a caricature. Perhaps microbiology students should be reminded (as physics students are) that investigators can themselves inadvertently interfere with the phenomena they are investigating.

Now let me take you to a scenario that developed recently at the West of Scotland Specialist Virology Centre in Glasgow, just a few miles north of the laboratory where the hapless veterinarian worked. Members of the staff there were using real-time PCR methods to evaluate throat swab samples sent in by primary medical centers participating in an influenza surveillance system embracing health boards in several different parts of Scotland. To their surprise, the Glasgow team found low-level influenza positive signals in 14 of the throat samples. Some were positive for a single influenza A or influenza B virus, while tests on other specimens indicated that more than one virus was present.

At that time, both influenza A and influenza B activities were very low in Scotland, no detec-
tions having been reported through monitoring schemes either there or in other parts of the United Kingdom and Europe. The team concluded, therefore, that the weakly positive signals did not reflect actual infections but might instead be a result of contamination from recent influenza vaccinations. Indeed, immunization with live attenuated influenza vaccine (LAIV) had been conducted over recent months in Scotland, and had for the first time included 2- and 3-year old children.

As Susan Bennett and her colleagues report in the *Journal of Medical Microbiology* (64:466, 2015), 7 of the 14 samples suspected of containing LAIV—including one which, on the initial influenza screen, was weakly positive for influenza B only—did indeed prove positive in a H2N2-specific assay. The remaining seven samples were negative, possibly because they had LAIV contamination at levels below the cutoff point of the assay.

“Of the 12 patients with a vaccine history, three were given inactivated influenza A vaccine (IAV) and the remaining nine had not received any influenza vaccination,” the authors write. “The two patients without a vaccine history were 29- and 75-year olds and therefore would not have been offered LAIV. Since none of the individuals were given LAIV, we cannot rule out the positives being a result of vaccine replication/shedding post vaccination. The false-positive results may be due to transmission of LAIV from recent vaccinated individuals as previous studies have shown that live vaccine can be, albeit rarely, transmitted between those given vaccine and close contacts.”

Could it be that intranasal administration of LAIV might allow large quantities of influenza A and influenza B viruses to become aerosolized, thereby contaminating the local environment? This might mean that patients with respiratory infections, entering that environment, would be unwittingly infected by the live, attenuated viruses. In an attempt to settle the question, Bennett and her coworkers got in touch with each of the primary health centers that had been conducting LAIV immunizations on site. All of the centers said it was indeed likely that both vaccinations and sampling had occurred in the same room. This finding mirrored the outcome of a previous investigation elsewhere which revealed the detection of IAV in environmental samples from areas where immunization clinics had taken place, and that vaccine could be recovered from surfaces for up to 66 days afterwards (T. Curran et al., J. Med. Microbiol. 61:332, 2012).

While reporting their findings, the Glasgow virologists issue a warning that laboratories using highly sensitive real-time PCR techniques should be conscious of the risk of LAIV contamination occurring within clinical samples taken at times when vaccination programs are running. Moreover, they say, public health bodies ought to be aware of this problem when they interpret surveillance data.

“Laboratories should consider using a LAIV-specific assay to confirm weak influenza A or B positive results, especially those detected concurrently with vaccination,” they write. “Using such a test will ensure that this type of contamination will not affect public health surveillance data or patient management and will prevent unnecessary laboratory expenses should such results be mistaken for PCR contamination.” They even find it necessary to warn centers that offer flu immunization to take particular care to decontaminate their environment before sampling patients with respiratory illnesses.

As with my story about *Brevibacterium otitidis*, it’s worth emphasising that the Glasgow incident did not occur in a temporary medical center in Nepal, or a hard-pressed field hospital in Ethiopia. Nor was it something that took place in a laboratory where early virologists were learning their craft, and taking substantial but unknown risks, in the first half of the 20th century. The incident happened in the present day, in a well-established, well-equipped facility staffed by highly trained, highly qualified, highly responsible microbiologists. Vigilance can never be set aside.