Labor Dr. Merk und Kollegen - Viruses under control

It is difficult to imagine how Ochsenhausen-based Labor Dr. Merk und Kollegen (LMK) would be able to develop, produce and test medical products for bacterial and viral contaminations without using cell cultures. The medium-sized company also produces viruses for testing and has stored more than 80 different viruses – enveloped and non-enveloped ones, animal and human pathogens – at -80°C.

Companies and institutions that test pharmaceutical manufacturing processes and medical products for the presence of viral contaminations or produce inactive pathogens for use in vaccines depend largely on the use of cell cultures. Dr. Ingrid Rapp, virologist and LMK's managing director, is very well aware of this: "Viral pathogens can only be produced in cell cultures," said Rapp explaining that viral parasites, which have a size of between 20 and 100 nanometres, do not have an own metabolism and can only replicate inside living cells.

Although the presence of viral pathogens can also be detected using PCR (polymerase chain reaction), this molecular biology method is nevertheless unable to differentiate between living and dead viral specimens of which only fragments are available. Ingrid Rapp pointed out that cell culture technology is indispensable for the production, identification or testing of infectious viruses.

LMK therefore offers combined virus cell systems that can detect different viruses. The company also produces special cell systems for the detection of specific viruses if required by clients.

Cells as indicator system

Dr. Ingrid Rapp, virus specialist.
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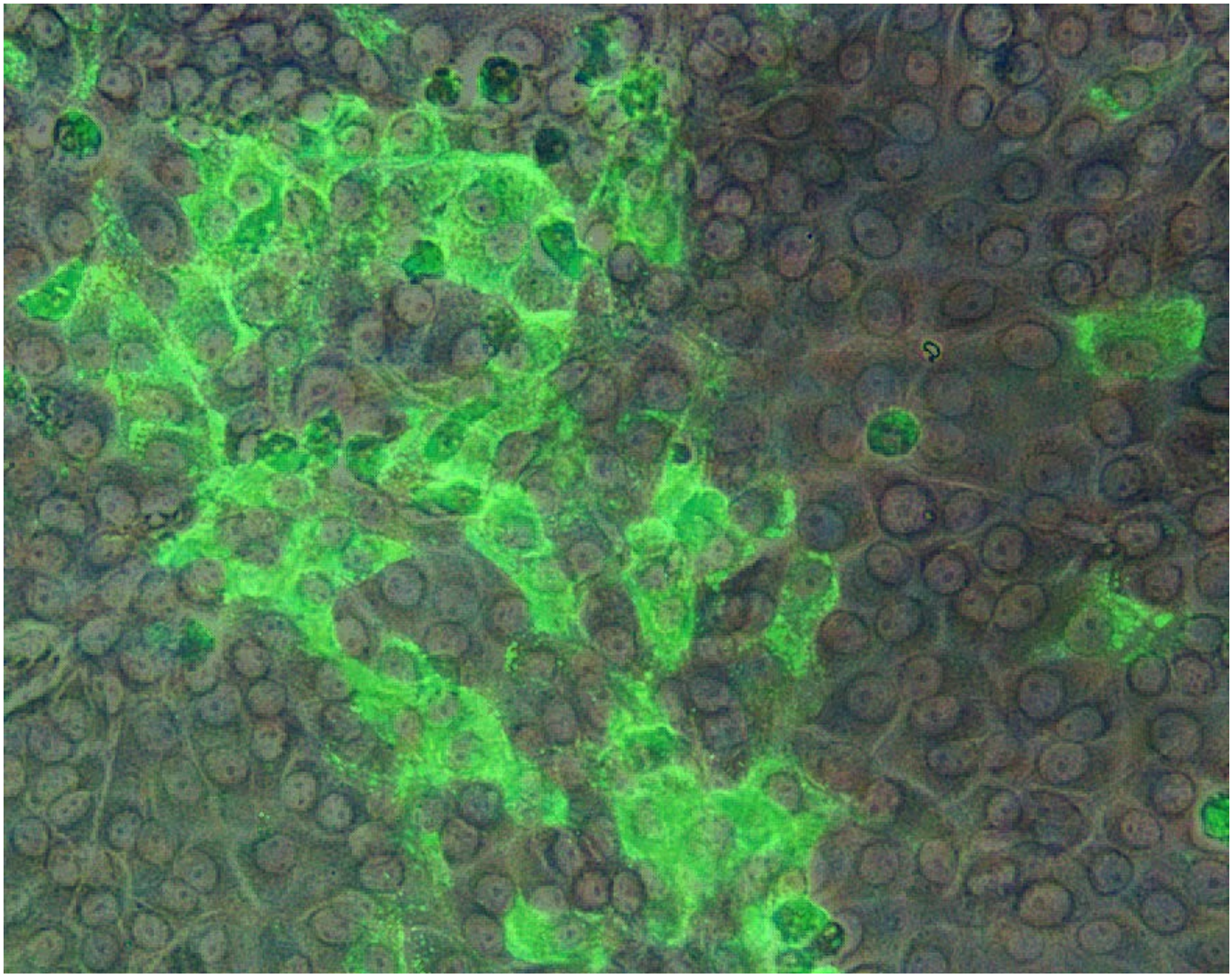
LMK offers tests for finding out whether disinfecting agents are able to inactivate, i.e. kill viruses. This works as follows: the disinfectant is incubated along with viral test strains and cultured for an appropriate period of time. All viruses that have survived the procedure will propagate and be detectable. The difference in the number of viruses (viral load) before/after the procedures indicates how effective the disinfectant under investigation is. In scientific terms, viral load is described as "log" (logarithm to base 10) and indicates the viral load changes after treatment. "Disinfecting agents must be tested with specific indicator viruses. These particular viruses cover the biochemical range of as many pathogens as possible, i.e. enveloped and non-enveloped, very resistant and barely resistant pathogens as well as DNA and RNA viruses," explains Ingrid Rapp. The development of a



Cell culture infected with rabies viruses. The image consists of two superimposed images – one light microscopy and one fluorescence image.
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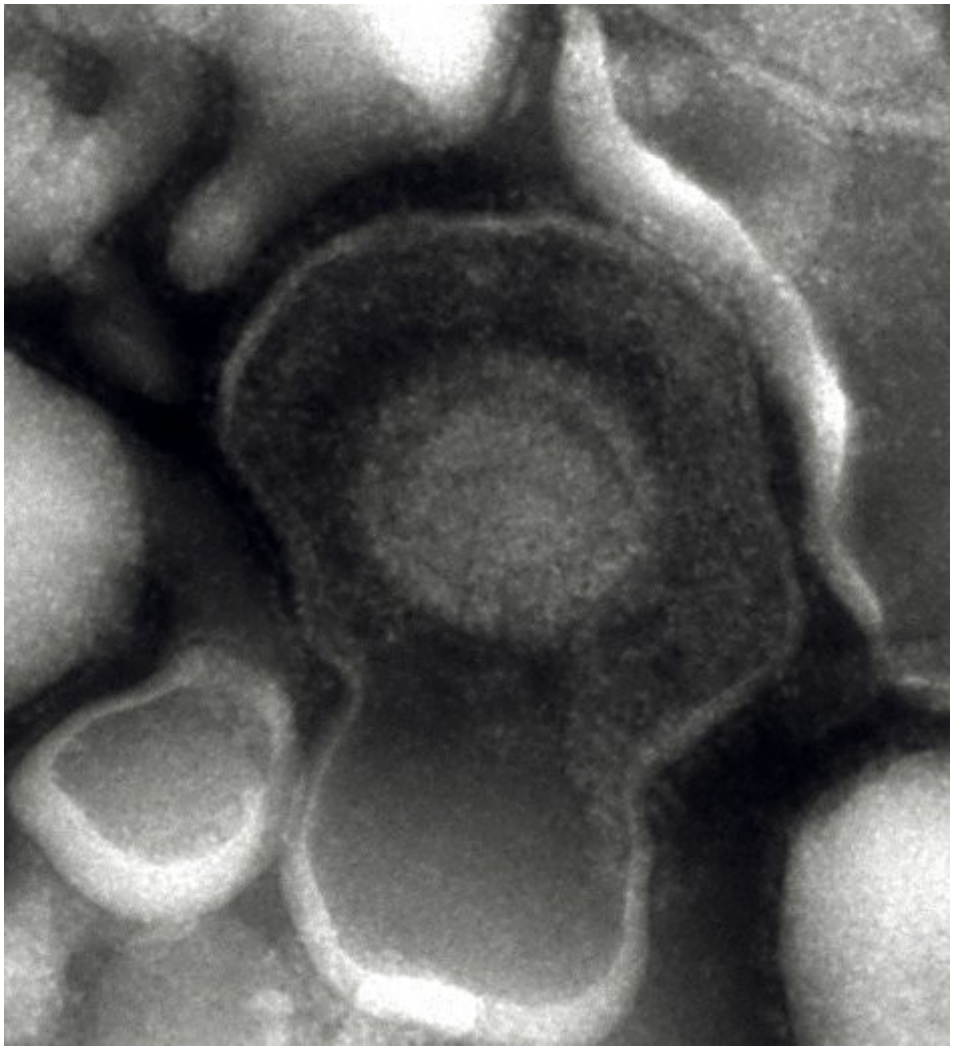
disinfectant involves testing out dozens of different substances.

Animal material can also be tested for viral contamination. This is of particular importance in the pharmaceutical industry where any animal material must be checked for contamination prior to production, for example when antibodies are produced from bovine plasma. LMK managing director Werner Dangel explains that the manufacturer of such a product must be able to decrease the viral load, either by killing the viruses or eliminating them by way of filtration. The test works as follows: test viruses are added to the animal material, which is then treated using the virus-elimination method implemented in a manufacturer's protocol. Subsequently, cell cultures are prepared to assess the efficiency of the used method in decreasing the number of viruses. For example, cell cultures can be applied to evaluate the virus-elimination efficiency of nanofiltration to find out whether the membrane pores are small enough to retain parvoviruses, which are very small non-enveloped viruses: a manufacturer provides LMK with a solution, which LMK enriches with a relatively high number of specific parvoviruses prior to nanofiltration. The filtered solution is then used in cell cultures to determine the efficiency of the virus-elimination method in reducing the initial virus load.

However, biopharmaceutical producers can contract LMK at a much earlier stage, for example when they experience problems with their cell cultures, such as cells that have stopped producing a sought-after active ingredient or are about to die. Biopharmaceutical producers contract LMK's "detectives" to help them identify the problem, as such work requires the expertise of cell and virus

specialists. LMK offers test systems that are able to solve around 70 per cent of cell culture-related problems.

26 cell systems – from simian kidney cells to mouse tail cells

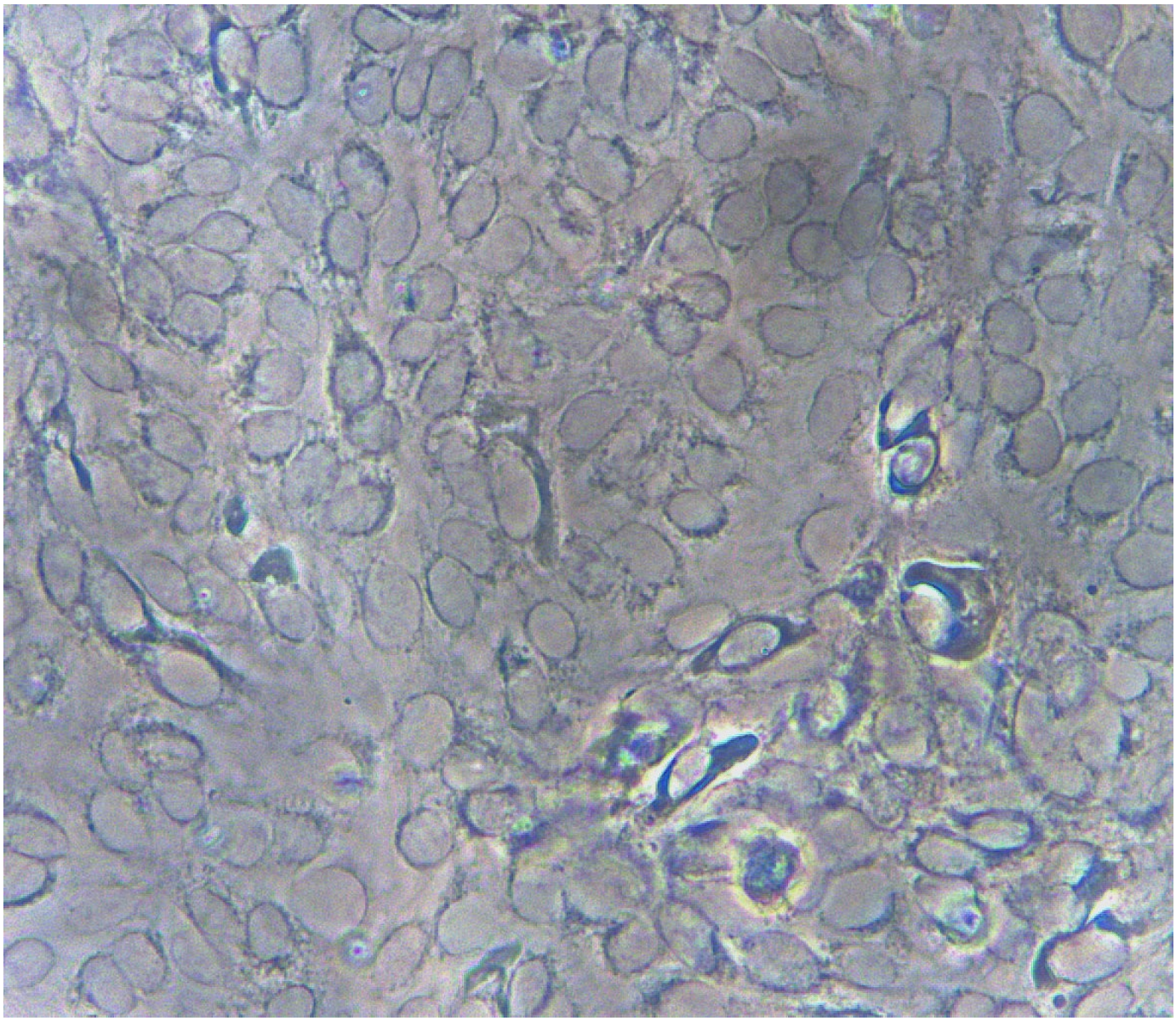


Varicella zoster virus, which causes chicken pox in children and Herpes zoster (shingles) in adults.

Biopharmaceutical safety tests that seek to detect viruses are procedures that consist of numerous steps (LMK also tests for microbial pathogens) and require a large number of viral strains, including human pathogens (hepatitis A virus, influenza A and B, rotavirus) and animal pathogens such as SV49, bovine parvovirus or vaccinia virus. The latter are also used as indicator viruses for human viruses and are grown in species-specific cell systems. At present, LMK has 26 cell systems, all of which are derived from mammalian cells with the exception of the SF9 insect (moth ovarian cells) cell line. LMK's cell systems are derived from 11 species, including human cells such as lung fibroblasts or lung carcinoma cells, porcine kidney cells (PK13), simian kidney cells (CV-1) or mouse tail cells (MA-23).

When research institutions and companies purchase a virus (a permit is of course required for doing so), the vendor provides information on the cell system that is best suited for culturing a specific virus. However, the culture is much more likely to be successful when the people in charge are highly experienced in working with viruses and cell cultures. Ingrid Rapp, for example, has around 30 years of experience in the handling of viruses behind her. Such expertise tends to come from working with viruses and exchanging information with other colleagues. Textbooks are unable to confer the

experience needed for the cultivation of viruses in cell cultures. Relatively simple viruses can be purchased along with the cells required for culturing them; they grow well, lead to high titres, can be easily frozen and thawed. “There are also viruses that do not grow well, and they have to be pampered,” said Ingrid Rapp explaining that some viruses have difficulty growing and propagate rather slowly in cell cultures, or indicator systems, which is the term used by virologists. Viruses can have different damaging effects on cells, which can be discerned under the light microscope. For example, poliovirus (which is cultured in simian kidney cells) destroys the entire cell lawn (adherent, confluent layer of cells in a culture dish); herpes viruses lead to holes (plaques) in the cell sheet and virtually inflate their host cells after a few days of incubation. It is highly important to be able to monitor and tell apart all these virus-specific morphological alterations.



Reoviruses that have infected Vero cells. The Vero lineage is derived from kidney epithelial cells extracted from the African green monkey.

Upon receipt of a reference virus, LMK propagates the virus in a cell culture and establishes subcultures (in a process known as passaging) for subsequent use. In general, the third subculture is used for laboratory work, the first is kept as a back-up and the second is used when the third subculture is no longer viable. In contrast to other cells that must be kept at -196°C in a nitrogen-gas phase (to prevent contamination), viruses can be stored at -80°C. Ingrid Rapp has gained

comprehensive expertise from the numerous projects she has conducted and she has also contributed to improving cell culture systems. She has shown, for example, that the Varicella zoster virus (VZV), which is cultured in human lung fibroblast cells, is not secreted by the host cells. This has implications for the propagation of the viruses: the host cells need to be repeatedly inoculated with viruses and infected cells need to be scraped off during passaging. In contrast, polioviruses can be transferred to new vials with the supernatant.

The viruses generate morphological changes in their host cells, and specific methods need to be used to recognise such changes. Rapp uses fluorescent antibodies to reliably achieve positive or negative results. Rapp highlighted that virus-specific host cell changes that are more difficult to discern require highly trained personnel. In addition, cell cultures are also the method of choice for determining the yield of active vaccine.

How do cells react to implants?

Toxicity testing of medical products also involves the use of cell cultures. LMK uses highly sensitive cell systems to evaluate the toxicity of implants and assess their tolerability. An example of this is a test which measures the quantity of substances being given off by an implant. The implants are exposed to a solution that mimics the human body as closely as possible and gradually give off substances. Cell cultures are then established and the cells are monitored for potential changes in behaviour, i.e. whether they die or propagate. Cells that are not exposed to implant-related substances are also monitored as a control. Thus, the cells' metabolic activity and proliferation rate provide important information on the toxicity of medical products. Cell culture tests carried out according to European and international standards have been shown to be excellent alternatives to animal experiments.

Cell cultures are used in in-vitro diagnostics and they are indispensible for determining the titre of inactivated viruses in vaccines as part of controlling the quality of vaccines. LMK supplies laboratory physicians with systems that are able to detect antibodies in the blood. However, Rapp knows from experience that cell cultures are less popular with laboratory physicians than they used to be, due to the fact that it is a relatively complex process that is time-consuming and requires a lot of experience. Cell culture methods are increasingly being replaced by molecular biology (PCR) or immunological methods.

LMK also produces viruses such as rotavirus, which only propagates slowly, in 60-l reactors. Rapp told us that this is what makes the company a leader in Europe and is why the company's expertise has become widely known, which is why many in-vitro diagnostics manufacturers contract LMK for development projects.

Article

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The article is part of the following dossiers

Cell culture technology: it all started with frog nerve fibres

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