

VIRAL VACCINES PRODUCTION

Pioneering role of Croatia and Institute of Immunology in production and quality control of vaccines

Institute of Immunology is the institution with a long tradition of production of viral vaccines, dating back to 1893 when the production of our smallpox vaccine began at the institution *Royal Institute of Animal Vaccine Against Smallpox in Zagreb (1893.)*, which was the predecessor of today's Institute of Immunology, in order to enable the implementation of the law on mandatory vaccination, with aim of smallpox eradication by using vaccine produced at the Institute of Immunology.

All of these data show us the important role of Croatia in the production of viral vaccines since the 19th century. It is a very little-known fact that the Croatian physician Ferdinand Hadvig has performed the first vaccination against smallpox in Jastrebarsko in Croatia even 5 years before the first historical vaccination against smallpox performed by Edward Jenner, who introduced vaccination against smallpox into medicine and is considered as the father of vaccinology.

Institute of Immunology has been producing numerous viral and bacterial vaccines during the 20th century, and has been the collaborative center of World Health Organization (WHO) for the research and development of immunobiological preparations; the collaborative center of World Health Organization (WHO) for bacterial vaccines and immunization programs and for the establishment of the international standard for immunobiological preparations as well as the institution that recruited the World Health Organization (WHO) experts and performed their education.

The role of the Institute of Immunology in promoting the importance of vaccination

Nowadays, the occurrence of campaigns against vaccination is becoming more often trying to convince parents that vaccination of their children is not necessary, which reduces vaccination coverage in the population and in turn increases the risk of epidemics of infectious diseases.

Institute of Immunology promotes the importance of vaccination by its continuous activity:

- vaccination is one of the greatest achievements of modern medicine
- the aim of vaccination is to reduce or eliminate the risk of serious diseases
- vaccination is one of the most effective measures to prevent getting infectious diseases
- vaccination is one of the public health interventions that has significantly contributed to reducing mortality from numerous infectious diseases.

Revitalization of the Institute of Immunology – strategic project of the Republic of Croatia

- Croatian Government has made the Conclusion regarding the revitalization of the Institute of Immunology, the institution with more than 130 years of tradition in manufacturing vaccines and other biological medicinal products.
- Presently the revitalization of production of viral vaccines, human plasma products and viper venom antiserum takes place.
- The revitalization includes the construction of the new production facilities for all the above-mentioned biological medicines with included viral vaccines production plant. Start of construction of the viral vaccines production plant is planned at the end of 2026 after the process of public bidding, with predicted start of production during 2028/2029.

About the viral vaccine strains used for the production at the Institute of Immunology

For many years the Institute of Immunology produced monocomponent and multicomponent live, attenuated viral vaccines that were present on the market of more than 100 countries around the world.

Currently, the Institute of Immunology stores Master seeds of measles, rubella and mumps owned by the Republic of Croatia and from which, as the final result of the revitalization process, viral vaccines will continue to be produced after the re-establishment of the production in new production facilities.

Viral vaccine strains used for the production of Master seed of measles virus Edmonston-Zagreb and Master seed of rubella virus RA 27/3 were used for the production of measles vaccine and rubella vaccine:

Measles virus vaccine strain Edmonston-Zagreb

- Is internationally acknowledged vaccine strain with long term use in vaccination programs in numerous countries
- Is documented in scientific literature as part of successful measures to control the spread of measles (*WHO/EPI/GEN/93.17*)
- Is owned by the Republic of Croatia

The first registration of the Measles vaccine in Croatia was performed in 1968. During the same year mandatory vaccination against measles was introduced.

- The World Health Organization (WHO) states that live attenuated measles vaccines develop a long-lasting immune response with detectable antibodies years after vaccination with these vaccines (World Health Organization, WHO Technical Report Series, No. 840, 1994. Annex 3)
- According to data from the World Health Organization (WHO), in comparative analysis of the use of different vaccine strains of measles virus, it is shown that the Edmonston-Zagreb measles vaccine strain records favorable characteristics of the duration of immunity in countries where it was used (*WHO/EPI/GEN/93.17*)

Measles virus vaccine strain Edmonston-Zagreb that was used at the Institute of Immunology for the production of Measles vaccine and Measles containing vaccines was developed at the Institute of Immunology by academician Drago Ikić and his team in 1967 using additional adaptation of the original strain Edmonston-Musser.

Academician Drago Ikić was a visionary, a great scientist, initiator of development of huge number of vaccines and the founder of the Institute of Immunology.

- According to available scientific research, the aerosol administration of measles vaccine produced from Edmonston-Zagreb measles vaccine strain was tested and immunological responses and the duration immunity data were compared to other measles vaccine strains administered in aerosol form. The data show that the Edmonston-Zagreb measles vaccine strain had favorable duration of immunity characteristics. These results are relevant to the research context and also indicate the potential for aerosol administration of vaccines.

Rubella virus vaccine strain RA 27/3

- Is internationally acknowledged vaccine strain with long term use in vaccination programs in numerous countries and documented in scientific literature
- Is owned by the Republic of Croatia
- The first registration of Rubella vaccine in Croatia was performed in 1970.

Mandatory vaccination against rubella was introduced in Croatia in 1975.

RA 27/3 rubella vaccine strain was developed at the Institute of Immunology by additional processing of the original rubella virus vaccine strain that was originally developed in USA by dr Stanley Plotkin which is one of the globally most widely used rubella virus vaccine strains.

Measles, mumps, rubella vaccine

The Institute of Immunology Inc. had been producing Measles, mumps, rubella vaccine (MMR) for almost 40 years. The first registration of Measles, mumps, rubella vaccine (MMR) was performed in 1974.

The long-term use of live attenuated vaccines produced using the measles, mumps and rubella vaccine strains developed at the Institute of Immunology was an integral part of the national vaccination program in the Republic of Croatia and it has contributed to a significant reduction in measles, mumps and rubella cases over the past decades.

Technology for the production of Measles, mumps, rubella vaccine as well as parts of Master seeds of measles, mumps and rubella virus were taken over by Indian company Serum Institute of India Ltd. during the 1980-s and started to be used in its production of viral vaccines.

In recent history, the Institute of Immunology produced measles vaccine, rubella vaccine and combined measles-rubella vaccine (MR) using internationally recognized vaccine strains: the Edmonston-Zagreb measles virus vaccine strain and RA 27/3 vaccine strain of rubella virus.

Since 2013 the Institute of Immunology did not produce active pharmaceutical ingredients or final bulks of viral vaccines. Lyophilization of viral vaccines also was not carried out since then.

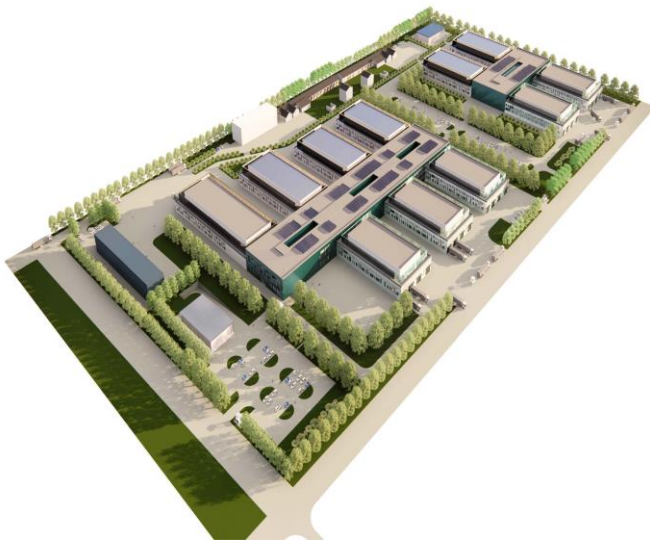
Vision of continuing the production of viral vaccines in new production facilities of the Institute of Immunology

There are assumptions for the restarting of viral vaccines production and quality control considering the fact that the Institute of Immunology has the production license, among other also for the specific parts of the viral vaccines production (storage of the cell cultures and Master and Working seeds of virus) and the GMP certificate for the certain addresses of production facilities where these activities are performed, which were obtained based on the regular supervision procedure performed by Agency for Medicinal Products and Medical Devices of Croatia (*Croatian: HALMED*) regarding the extension/renewal of the production license for the specific parts of the viral vaccines production.

Aseptic production of viral vaccines requires strict compliance with EU GMP and WHO standards. Therefore, a permanent solution and a basic prerequisite for the long-term sustainability of viral vaccine production at the Institute of Immunology and the potential placement of vaccines through WHO and entry into international markets is the construction of new production facilities that meet all regulatory and technological requirements, with all modular construction elements necessary for facilities for the production of viral vaccines, quality control and development.

The Center for Research and Knowledge Transfer in Biotechnology (*Croatian: CIPZB*) at the University of Zagreb is in charge, and the Institute's Research and Development Department is engaged to carry out activities related to the product research and development.

What are the reasons for investing in viral vaccines production at the Institute of Immunology?





Medicinal reasons for investing in production at the Institute of Immunology

- Measles remains among the leading causes of child mortality in underdeveloped countries.
- The use of live, attenuated vaccines is encouraged globally and remains as a key public health tool.
- The Institute of Immunology used internationally recognized vaccine strains of proven quality for the production of live attenuated viral vaccines.
- The long-term use of live attenuated vaccines produced using the aforementioned measles and rubella vaccine strains as an integral part of the national vaccination program in the Republic of Croatia, has contributed to a significant reduction in measles and rubella cases over the past decades.
- In the first phase, the Institute of Immunology will produce monocomponent vaccines against measles and rubella (1-dose, 5-dose, 10-dose) and the multicomponent measles containing vaccines (1-dose, 5-dose, 10-dose) thus covering a wide range of health system needs.
- The World Health Organization (WHO) states that live attenuated measles vaccines develop a long-lasting immune response with detectable antibodies years after vaccination with these vaccines (World Health Organization, WHO Technical Report Series, No. 840, 1994. Annex 3)
- According to data from the World Health Organization (WHO), in comparative analysis of the use of different vaccine strains of measles virus, it is shown that the Edmonston-Zagreb measles vaccine strain records favorable characteristics of the duration of immunity in countries where it was used (WHO/EPI/GEN/93.17)
- Live, attenuated vaccines have documented immunogenicity and are widely used in public health systems.

Economic reasons for investing in the vaccine production at the Institute of Immunology

- Viral vaccine production is a strategically important and globally growing sector.
- New production facilities designed in accordance with GMP and WHO requirements will allow expansion of production capacities, which will create significant market potential.
- The design of production facility allows for the parallel production of multiple types of vaccines, which significantly increases production capacity and flexibility in relation to market needs.
- The technology for measles and rubella vaccines manufacturing is based on an internationally recognized technological approach, comparable to practices applied in global vaccine production.
- Stable global demand indicates faster business results after the start of production.
- There is a long-standing interest from a number of countries in the vaccines of the Institute of Immunology, which confirms a sustainable export-oriented business model.
- Due to the increasingly frequent occurrence of epidemics, an increase in the need for vaccines is expected.
- The global vaccine market is growing rapidly, with vaccines for pediatric use dominating the international vaccine market.
- A significant trend of increasing pediatric vaccines coverage indicates the importance of the pediatric segment.

- By increasing the capacity of new production, which cannot be carried out at the existing location, the financial justification of the construction is ensured.
- With the new production facility in accordance with GMP demands, certified by WHO, we will be able to significantly expand the market for the placement of our products.
- Only in this way Croatia will provide for its needs at all times a sufficient quantity of the necessary medicinal products, from the product range of the Institute of Immunology, as well as new vaccines, which can be of particular importance in situations of shortage of medicines on the market, caused by increased demand for products due to e.g. natural disasters, wars or pandemics such as COVID-19, which would allow the Republic of Croatia to be independent on production in other countries.
- Many countries in South and Southeast Asia are just now introducing vaccination against rubella, which is the reason why a significant market is opening up for the sale of measles and rubella vaccine (MR), especially taking into account that these are the most populous countries in the world, with India being the most populous country in the world (1.47 billion inhabitants in 2025) with a continuously high natural increase in the population, which is the main reason why their need for vaccines, especially pediatric vaccines, is increasing.
- Bearing in mind the above-mentioned facts about the need for the measles and rubella vaccine (MR), need for the measles, mumps and rubella vaccine (MMR) as well as the monovalent measles vaccine which exceed the current global production possibilities, the placement of the number of vaccines that the Institute of Immunology intends to produce in the new production facilities is unquestionable.
- Institute of Immunology has registered combined vaccine against measles and rubella (MR), which is currently a hit in countries that are currently in the process of introducing higher standards of vaccination within the national vaccination program (e.g. countries of South and Southeast Asia) and are now switching from monovalent vaccines (Measles or Rubella) to the vaccine against Measles and Rubella (MR) and still these countries cannot afford the three-component vaccine against measles, mumps and rubella (MMR) for their mandatory vaccination, and that is the reason why a significant market is opening up for the sale of Measles and Rubella vaccine (MR), especially considering that these are the most populous countries in the world.
- Production of viral vaccines is extremely profitable and it has always been largely focused at the Institute of Immunology on export to foreign markets, considering that even with the previous production capacity and yields, quantities of vaccines that were produced significantly exceeded the needs of the Croatian market.
- After finishing construction of the new production facilities, production of CVPs of measles and rubella vaccine, final bulks and lyophilized products will be enabled with significantly higher annual production capacity, since lyophilization of viral vaccines will be carried out again in the new facilities.
- All the plans that were made include, in addition to monocomponent and bicomponent vaccines, also the MMR vaccine, which is currently in the process of development.

Technology for viral vaccines production

- The technology for measles and rubella vaccines manufacturing is based on an internationally recognized technological approach, comparable to practices applied in global vaccine production. This technological approach has been proven applicable in several countries and has been used in public health programs for a long time.
- Production of Measles vaccine, as well as of Rubella vaccine, is based on replication of virus on the cell substrate and collecting the virus harvests that have to be additionally stabilized and clarified. Produced harvests of virus and active pharmaceutical ingredients of vaccine (i.e. CVP - clarified virus pool) must be frozen at temperature -60 °C or below until finishing quality control testing and after that CVPs can be used for the preparation of final bulks of viral vaccines.
- Finished vaccine was being prepared from the final bulk of viral vaccine through the process of finalization (including filling and lyophilization). During the time when the production of viral vaccines was performed at the Institute of Immunology, after finishing quality control testing at the Institute of Immunology each vaccine batch was additionally tested by special quality testing at Agency for Medicinal Products and Medical Devices of Croatia (*Croatian: HALMED*).

- Production of active pharmaceutical ingredients of measles vaccine and rubella vaccine was based on the principal of virus banks (usage of Master seed of measles virus and Master seed of rubella virus as well as Working seeds of measles virus and rubella virus produced from these Master seeds) and cell banks (usage of Master cell bank of MRC-5 cells and Working cell bank of MRC-5 cells produced from the Master cell bank of MRC-5 cells). Working cell bank of MRC-5 cells was used for the preparation of production cell cultures that were used as substrate for virus propagation. Working seed of measles virus and Working seed of rubella virus were used for the inoculation of production cell culture of MRC-5 cells that was used as a substrate for the virus propagation.
- Using previously established virus banks and cell banks it was enabled that the production of vaccine was always performed with well characterized and tested starting materials.

Production of viral vaccines is based on the principles of Good Manufacturing practice (GMP, EC GMP Eudralex Volume 4) and it was performed according to:

- Requirements for Measles, Mumps, Rubella vaccines and combined Vaccines (Live). WHO Expert Committee on Biological Standardization. Forty-third Report. Geneva, World Health Organization, 1994 (WHO Technical Report Series, No. 840)
- Requirements for the Use of Animal Cells as *in vitro* Substrates for production of Biologicals, (Requirements for Biological Substances No.50) in WHO Expert Committee on Biological Standardization. World Health Organization, 1998 (WHO Technical Report Series, No. 878)
- Appropriate monographs of the current Ph. Eur. (European Pharmacopoeia)
- Standard operating procedures
- Internal demands

Development of new vaccines and technology platforms

- Through product development, improvements are continuously implemented through the introduction of changes in accordance with regulatory requirements
- Other ways in which vaccines are being further developed include the use of recombinant viral vectors used in modern research platforms.
- The Institute of Immunology is developing a new recombinant vaccine strain of the mumps virus with the aim of creating the foundation for the future development of a combined vaccine against measles, mumps and rubella.
- After completing the necessary development steps, the new candidate for a combined measles, mumps and rubella vaccine could represent a significant contribution to the product portfolio of the Institute of Immunology.
- The Institute of Immunology is involved in the development of the new platforms for the production of mRNA vaccines.

Eradication of measles and other diseases

In 2012 The Global Vaccine Action Plan by World Health Assembly was accepted with main goal of measles elimination in 4 WHO regions (of the total number of 6 WHO regions) until 2015. Recent reports show that the Global Vaccine Action Plan WHO was not realized since there are inconsistencies in implementing vaccine availability plan for all the children and measles were not eliminated in 4 regions of the total number of 6 WHO regions.

For that purpose, financial resources are needed as well as the commitment for the realization of this goal to reach out to every child and react to every occurrence of measles and prevent their spreading. One of the priority goals of WHO is achieving higher global vaccination against measles to at least 90 % or higher.

In 2020 it was planned that UNICEF would annually distribute around 200-250 million doses of measles vaccine, with the assumption that the need for the vaccines will be even higher that was planned due to the frequent occurrence of epidemics.

Although bearing in mind the goal of global measles eradication, national programs increasingly use a combined vaccine containing all three antigenic components (measles-mumps-rubella), still a good part of underdeveloped countries, supported by donations through international agencies, use a monovalent vaccine against measles.

In recent years, many countries in South and Southeast Asia have started introducing rubella vaccination, which opens up a significant market for the measles and rubella vaccine (MR), especially considering that these are the most populous countries in the world, with India being the most populous country in the world (1.47 billion inhabitants in 2025) with a continuously high natural population increase, which is why the need for vaccines, especially pediatric ones, is increasing.

Measles outbreaks in the world

Measles outbreaks are caused by irregularities in routine immunizations and in mass vaccination campaigns and it represents serious problem in numerous countries.

According to UNICEF, measles epidemics have occurred in over 100 countries around the world over the past five years.

According to UN data, measles epidemics are becoming more frequent, and in 2024, large measles epidemics were recorded in 59 countries, which is three times the number in 2021, with measles completely eliminated in quarter of these countries.

During 2015 great measles outbreaks appeared in Egypt, Ethiopia, Germany, Kyrgyzstan and Mongolia. Measles outbreaks in Germany and Mongolia spread even to the older population, showing that adolescents and adults may remain unprotected if they are not covered by vaccination programs. There is an increased risk of measles outbreaks in countries experiencing humanitarian crises due to the inability to achieve high vaccination coverage.

According to the CDC, measles epidemics have also occurred in economically developed countries over the past fifteen years in Denmark, Greece, Kenya, Poland, Spain, Sweden, Ukraine, Venezuela, and among adolescents and young adults in the United States, Canada, Australia, Mexico and United Kingdom.

WHO continuously collects data on the occurrence of measles in countries around the world and publishes them in the form of annual reports at the global level and at the level of individual regions of the world, in order to monitor trends in the occurrence of measles and collect important data for expert and advisory bodies (SAGE) that make decisions about the implementation of immunizations (e.g. whether additional doses of the vaccine are needed, the age at which the vaccine is administered, etc.).

WHO reports from November 2025 state that the number of deaths caused by measles decreased by 88% between 2000 and 2025, but almost 300 children still die because of measles every day.

According to World Health Organization (WHO) around 95 000 people, mostly children, died from measles in 2025. The WHO states that the continued high mortality rate is linked to insufficient and uneven coverage of routine vaccination in certain regions.

According to the estimations of UNICEF, WHO, GAVI and CDC their mass vaccination campaigns and global spreading of areas where the routine vaccinations against measles were performed saved approximately 20,3 million of lives between 2000 and 2015, but as the improvements were not consistence in all the countries approximately 20 million of children did not receive even a dose of measles vaccine in 2015.

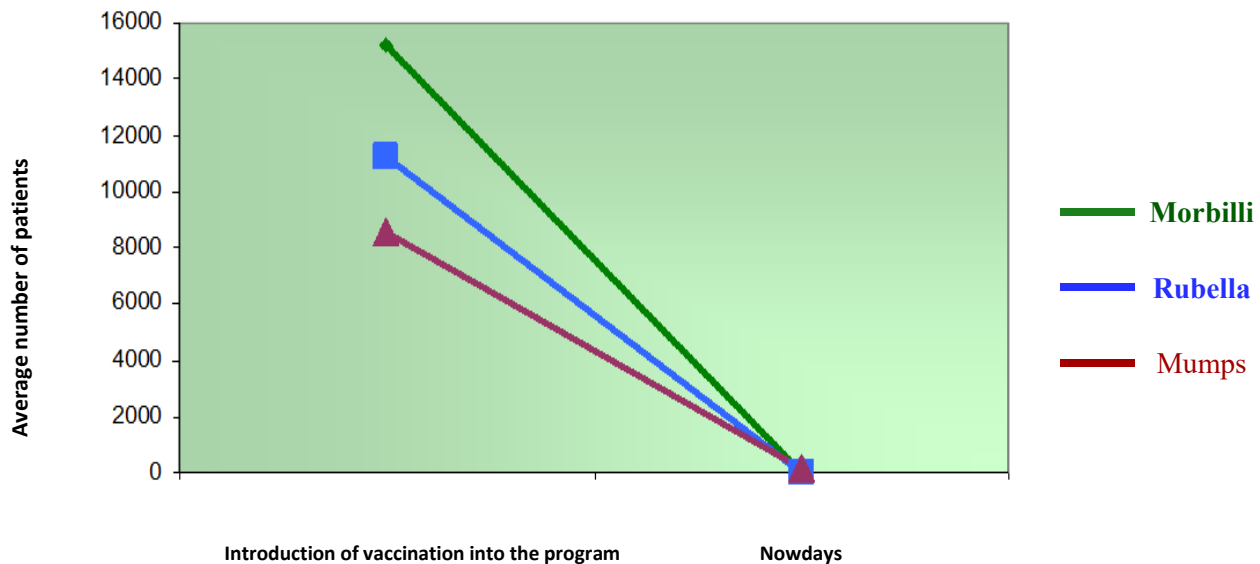
According to UNICEF estimates, since the introduction of measles vaccination in 1974, vaccination has saved almost 94 million lives.

According to WHO measles are the key indicator of the strength of each country's immunization system and the occurrence of measles outbreaks warns of difficulties in the functioning of the health system.

Vaccines produced by the Institute of Immunology have been distributed to numerous countries over the past decades, including countries facing increased measles incidence. During that period, individual countries expressed interest in using vaccines produced at the Institute of Immunology, which contained internationally recognized vaccine strains, including the Edmonston-Zagreb strain.

According to the Croatian Health Institute of Public Health, during the period of measles, mumps and rubella vaccination in the Republic of Croatia, a very significant decrease in the incidence of these diseases was recorded. Over a period of several decades, the incidence of measles and rubella was reduced for more than 99 %, and the incidence of mumps was reduced for about 98 % (Croatian Health Institute of Public Health, 2005). During that period outbreaks of measles and rubella were rare, while sporadic outbreaks, especially of mumps, occasionally occurred in areas with intensive tourist traffic, for example in Dubrovnik).

Reduction of measles, mumps and rubella incidence due to the vaccination with combined vaccine



Graphic representation of the reduction of measles, mumps and rubella (data from Croatian Health Institute of Public Health, 2005)

Due to the long-standing high vaccination rate in the Republic of Croatia, the incidence of measles, mumps and rubella has been very low for a long period. In recent years, there have been changes in the perception of vaccination among some parents, influenced by different information and attitudes about vaccination.

Market potential and export opportunities

- Vaccines produced at the Institute of Immunology have been marketed in more than 100 countries over the decades.
- Target markets for viral vaccines produced in future new production facilities of the Institute of Immunology will be the markets of the WHO and UNICEF programs, the markets of South and Southeast Asia and Latin America and Africa, given the long-standing interest of these regions in the Institute of Immunology's viral vaccines and the fact that they have been present on international markets, especially in South and Southeast Asia, for many years. Due to their long-standing recognition and global demand, competitiveness is also expected in the markets of South America and Africa.
- The global vaccine market is experiencing continuous and rapid growth, and the value of the global market has quadrupled in the last 20 years.
- Global trends in the field of vaccines are influenced by demographic factors including population growth and aging, as well as technological advances in vaccine development and production.

For example, countries with high birth rate, such as India and China, plan for higher pediatric vaccine doses in line with their national immunization programs. The same applies to countries with lower birth rates, including European Union countries, which define their pediatric vaccine needs according to current immunization schedules.

Analyses also indicate increased interest in immunization across different age groups, including the adult population.

According to the available analysis, the global vaccine market is influenced by various factors, including the concentration of production capacity in a smaller number of large manufacturers, requirements for ensuring an efficient cold chain during distribution, and the increasing need for transparency of information about vaccine development and production processes.

It is also stated that the attitudes of a segment of public expressing skepticism toward vaccination may affect the demands for vaccines in certain communities.

The safety of pediatric vaccines is also discussed in public sphere, partly fueled by misinformation about side effects. One of the most frequently cited examples is an article published in 1998 in the journal *Vaccine*, but which was soon retracted due to the unfoundedness of the claims made in this article about the existence of a connection between the MMR vaccine and autism in some children, since numerous scientific studies have been conducted that have confirmed that there is no connection between the MMR and autism.

According to publicly available guidelines from international health organizations, live attenuated vaccines play an important role in immunization programs in many countries and their use is encouraged globally.

Key reasons for starting production – preventing outbreaks of measles, mumps and rubella

Medically positive effect of vaccine production at the Institute of Immunology for the Republic of Croatia

- ensuring the supply of viral vaccines for the healthcare system
- research and development of vaccine and other medicines

Positive economic effects of vaccine production at the Institute of Immunology for the Republic of Croatia

- the possibility of ensuring further development and supporting professional knowledge
- new production facilities can have positive effects on the national biotechnology industry
- possibility of reducing additional costs for the healthcare system

Strategic positive effects of vaccine production at the Institute of Immunology for the Republic of Croatia

- retaining highly educated personnel in the Republic of Croatia
- preserving our own production will, in the long term, provide an incentive for the further development of biotechnology in Croatia and provide a chance for highly skilled young people to find employment in their own environment.

Investing in such infrastructure also represents a mechanism for strengthening the resilience of the biological medicinal products supply system, especially during the periods of global disruptions in supply chains.

According to the World Health Organization, the measles virus is considered one of the most contagious human viruses, which is why the continued availability of vaccines is an important component of planning public health measures.

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