



# Drago Ikić – portrait of a renowned Croatian vaccinologist

## Vision, mission, strategy and goals

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Drago Ikić spent most of his career studying principles of vaccine production, safety and effectiveness with an eye toward biological standardization and quality assurance.

Key principles of his efforts were to:

- Develop new and improved vaccines and enhance vaccine safety by building quality into the product in the first place.
- Increase national and then global prevention of death and disease through safe and effective vaccination.
- Institute training on the job and self-improvement.
- Institute international and national education program.
- The need to know more about everything in the system

Drago Ikić was born on July 2, 1917. In 1936 he attended the School of Medicine, University of Zagreb, earning an M.D. diploma in 1942, a M.Sc. degree (Hygiene) in 1952, a M.Sc. degree (Bacteriology) in 1954, and a Ph.D. degree in Immunology in 1958. His management skills and performance were proven through numerous posts. Between 1952 and 1956, he chaired the Department for Control and Research of Vaccines at the Central Institute of Hygiene in Zagreb, he ran the Institute for Control and Research of Vaccines in 1956 and from 1958–1961, and directed the Serovaccinal Institute in Zagreb from 1956–1958.

In 1961 Drago Ikić founded Institute of Immunology Zagreb. The Institute of Immunology continued, like its predecessor Serovaccinal Institute, with the research, development and production of immunological drugs and medicines derived from animal and human blood. Drago Ikić's vision was that the Institute of Immunology should be a science-based organization that produces and regulates biological products, by helping stakeholders within the public health system in fighting infectious diseases (preventive medicine), in treating diseases with immunological drugs and medicines, and in increasing awareness of the importance of continuous quality improvements. In order to respond to these challenges in the best possible way, experts from the Institute of Immunology, under the leadership of Drago Ikić, have developed their competences by learning from the best European and global examples of good practice and their own experience in quality assurance procedures. Drago Ikić has initiated and actively participated in a wide range of activities aimed at changes and reforms in the field of quality assurance in public health, research (natural sciences, biomedicine and biotechnology) and in higher education.



**Figure 1.** On July 28, 2012, the President of the Republic of Croatia, Ivo Josipović, presented awards to deserving persons. Drago Ikić (second on the left) was awarded the Order of Danica Hrvatska with the face of Ruđer Bošković for outstanding contribution to science (<http://www.predsjednik.hr/lipanj96>).

Though Drago Ikić officially retired in 1982, he never stopped working and continued to be industrious. As Fellow of the Croatian Academy of Sciences and Arts (former Yugoslav Academy of Sciences and Arts) from 1977, he founded in 1983 as part of the Department of Medical Sciences, the Institute (nowadays Cabinet) for Research and Standardization of Immunological Substances and become its titular director. His research focuses on immunologically active substances in viral precancerous, cancerous, and other diseases. He continues to cooperate with other scientific institutions at home and abroad. An important part of his recent research was the application of various interferons in head and neck tumors and melanoma, as well as in precancerous and cancerous conditions of the cervix uteri.

Drago Ikić is the author of over 225 scientific papers and reviews, editor of many books and proceedings (listed on his personal page at [http://info.hazu.hr/regular\\_members](http://info.hazu.hr/regular_members)). Headed by Drago Ikić, the Institute of Immunology has organized eighteen international meetings (mainly cosponsored by the Academy of Sciences and Arts) resulting in 14 published proceedings in English. The meetings and published material cover the following fields of interests: biological standardization (1957), immunology (1959), microbiological standardization (1960), characterization and use of human diploid cells (1963, 1970), biological standardization of cell cultures (1964), oncogenicity of viral vaccines (1968), small pox eradication (1969), acute respiratory diseases (1969), influenza vaccine (live) (1971), bacterial vaccines (1971, 1977), combined vaccines (1972), field trials of

vaccines (1973), production, standardization and clinical use of interferons (1975, 1977, 1979), effectiveness and stability of vaccines against measles, poliomyelitis and pertussis (1976).

Drago Ikić's remarkable contributions to the field of microbiology and vaccinology in Croatia were repeatedly awarded. Among his many accolades he received the »Ruđer Bošković« award in 1972, a Life Achievement award in 1980 from the Republic of Croatia, and Order of Danica Hrvatska with the face of Ruđer Bošković in 2012 (Figure 1). However, despite his numerous awards and prestigious accomplishments, Drago Ikić continues as an extremely humble and down-to-earth man.

## BACTERIAL VACCINES

Drago Ikić's research created a new scientific paradigm for development of live, attenuated virus vaccines. But, in the mid 1940s, due to epidemic outbreaks of diphtheria and typhoid fever, he began research on bacterial vaccines. At the brink of the 20th century, live vaccines were being used worldwide for smallpox and rabies, heat-killed vaccines for cholera, typhoid, and plague, and antitoxins for diphtheria and tetanus. Drago Ikić found the prospect of being able to apply these discoveries to prevent and treat these dangerous diseases very exciting. Between 1946 and 1952 he completed his training in bacteriology and hygiene in well-known European laboratories (Paris, Zurich, and Geneva) and studied principles of vaccinology in London and Copenhagen.

After World War II the need arose for organized, well planned and controlled field trials on bacterial vaccines and in 1950s he joined the Yugoslav Typhoid Commission. In 1953, the Yugoslav Typhoid Commission organized the first strictly controlled field trial of two types of anti-typhoid vaccine in an attempt to determine the relative and absolute effectiveness of each. The preliminary report (1) published in 1957 has given the basic information on the conditions of the trial, in which 35 508 persons completed the course of two injections. The members of the Commission were: Branko Cvjetanovic (School of Public Health, Medical Faculty, University of Zagreb); Hinko Emili (Chairman) (Central Institute of Hygiene, Zagreb); Drago Ikić (Central Institute of Hygiene, Zagreb); Ante Merdžo (General Hospital, Department for Infective Diseases, Osijek); Fran Mihaljević (Fever Hospital and Department of Infectious Diseases of the Medical Faculty, University of Zagreb); A. Nevidal (Institute of Hygiene, Osijek); J. Rucker (Central Institute of Hygiene, Zagreb) and Pavao Tomašić (Central Institute of Hygiene, Zagreb). Drago Ikić supervised laboratory studies and the protective value of heat-killed phenol-preserved typhoid vaccine was confirmed (Figure 2).

Late in the 1950's; Drago Ikić felt that the interest in bacterial vaccines slackened and many have been attracted by the development of virus vaccines. At the symposium on bacterial vaccines held at the Institute of Immunology in 1971 he proposed »a renaissance of bacterial vaccines«. Further improvement of bacterial vaccines in his view should have followed two trends: the development of live, i.e. oral vaccine and the separation and concentration of the active substance from the germs that

have been used for pneumococcal and meningococcal vaccines and vaccines against *H.influenzae* infections. Drago Ikić believed that oral vaccine is convenient for mass prophylaxis of all diseases because of simple mode of administration, the imitation of natural infection, and the exploitation of local defence mechanisms, provided that the protection offered by it is reasonable and the vaccine harmless (2). His belief differed from the existing dogma that vaccines made from killed or inactivated microorganisms are safer than attenuated vaccines, which are made from weakened, but live, microorganisms. Also, there is a small chance that an attenuated vaccine might cause the disease it is designed to prevent. Although first attenuated vaccine was developed in 1885 when Pasteur developed a vaccine against rabies based on live attenuated virus, the development of the first heat-killed vaccine against cholera by Theobald Smith and Daniel Elmer Salmon (3) a year latter was a major step in vaccine development. The original work on heat-killed vaccines proved to be highly valuable and over the next 20 years led to the development of killed vaccines for several human infectious diseases—typhoid, cholera, and plague. It was not until 1927 that the following live vaccine was developed: the bacille Calmette-Guérin (BCG vaccine) against tuberculosis. Then after World War II the ability to make cell cultures, i.e. the ability to grow cells from higher organisms in the laboratory, made it easier to develop new vaccines and the number of pathogens (mostly viruses) for which vaccines could be made almost doubled. Despite his confidence that the development of new and better bacterial vaccines is epidemiologically ever more justified, Drago Ikić has slowly changed the focus of his interest.



**Figure 2.** Drago Ikić (second on the right) speaking at working meeting of experts on bacterial vaccines and standards at Andrija Štampar School of Public Health, Medical School of the University of Zagreb in 1959.

## VIRAL VACCINES

Vaccine research flourished because of new techniques for growing viruses in tissue culture. Late in 1950's Hayflick and Moorhead (Wistar Institute, USA) began their work on human fetal fibroblasts (4). It soon became evident that such cultures were susceptible to human viruses, were diploid and had a finite life time. The finite lifetime of cultured, normal human and animal cells is a reflection of the maximum capability of that cell lineage to replicate. They can be frozen and stored in liquid nitrogen at an early passage level, recover from frozen ampoules years later and induced to divide again. One such strain called WI-38 was the most widely used and highly characterized normal human cell population in the world. In September 1966, J.P. Jacobs (Medical Research Council Laboratories, London) developed, stockied and characterized another human diploid cell strain, also derived from fetal lung tissue, MRC-5 for vaccine manufacture. From the very beginning Drago Ikić was deeply involved in the research and development of human diploid cell strains. At a meeting held in 1963 in Opatija (proceeding was edited under direction of the Institute of Immunology Zagreb), Drago Ikić and collaborators had nine presentations on poliovaccine and measles vaccine produced on human diploid cells (5). The most prominent scientists in the field attended the meeting (L. Hayflick, H. Eagle, J.P. Jacobs, H. Koprowski, P.S. Moorhead, F. Perkins) and Drago Ikić established the opportunity for continuous international collaboration. Later, at the meeting held in October 1968 in Zagreb (6), three problems were considered regarding the propagation of human diploid cells for use in preparing viral vaccines for man: virally transformed cells, the presence of oncogenic viruses and the presence of contaminating non-oncogenic viruses or other organisms such as mycoplasmas. In considering these problems, general principles for selection of a diploid cell substrate for viral vaccines were suggested and published in *Science* – and Drago Ikić had his place among distinguished experts (7).

Since the development in 1961 of a human diploid cell strain (HDGS) culture system for isolating viruses, HDGS-derived human vaccines have been licensed worldwide for polio IPV and OPV (multiple strains); rabies (Pitman-Moore L503 3M strain); rubella (RA27/3 strain); measles (Edmonston-Zagreb strain); varicella-zoster (Oka strain); mumps (Rubini strain) and hepatitis A (HM-175, CR-326-F, and GBM strains). Many of these widely used vaccines now have at least a 30-year record of safety after extensive, ongoing pharmacovigilance.

The first candidate poliovaccine, based on one serotype of a live but attenuated (weakened) virus, was developed by the virologist Hilary Koprowski. Koprowski's prototype vaccine was given to an eight-year-old boy on February 27, 1950. Koprowski continued to work on the vaccine throughout the 1950s, leading to large-scale trials in the then Belgian Congo, Croatia and in Poland against serotypes PV1 and PV3 between 1958 and 1961. Recently, Koprowski has described his experience in collaboration with local scientists and wrote (8): »Another per-

son who appeared on the scene at this time was Drago Ikić, head of the Immunology Institute of Zagreb in the former Yugoslavia. Once decided, he carried out projects meticulously. So it was when he decided to vaccinate Croatia with our types 1 and 3 strains. In early spring of 1961, over 1,300,000 children in Croatia were given a mixture of the two strains (9) and were controlled clinically and serologically. No postvaccination polio was seen and the serologic studies suggested that there were at least 100,000 triple seronegatives in the vaccinated population. The data showed a seroconversion of 91.5% for type I and 93.5% for type 3 (10, 11). Later, Ikić produced attenuated poliovaccine locally using human diploid cells and became well-known for his use of these cells in vaccine production (11, 12).«

The area of poliomyelitis vaccine development was very competitive. The second vaccine, developed in 1952 by Jonas Salk, was inactivated poliovirus vaccine (IPV) based on a poliovirus grown in monkey kidney tissue culture (Vero). Albert Sabin developed another live, oral polio vaccine (OPV). Human trials of Sabin's vaccine began in 1957, and in 1958 it was selected, in competition with the live vaccines of Koprowski, and in 1962 became the only polio vaccine used worldwide. However, on very rare occasions, the attenuated virus in OPV reverts into a form that can paralyze. Recently, most industrialized countries have switched to IPV, which cannot revert, either as the sole vaccine against poliomyelitis or in combination with oral polio vaccine.

Preparation of vaccines for other viral diseases at the Institute of Immunology soon followed, including measles, mumps, rubella (German measles), and rabies. Vaccine development is a long process, with the time from early research to licensure steadily increasing. At one time the process took about ten years; now it takes closer to fifteen to twenty years. The process begins with fundamental research in universities or biotech firms who have an idea. However, to take things further, there must be a vaccine manufacturer, a regulatory authority ready to give permission for the use of vaccine (licensing), and public health authorities that will recommend and foster vaccination. In 1963, when he started with development of Edmonston-Zagreb measles vaccine strain, Drago Ikić had it all. The original seed virus was obtained from Musser laboratory as the Edmonston-Musser strain (Research and Development Laboratories, Philips&Roxane Inc., USA) with a history of 24 human kidney, 28 human amnion tissue culture passages, 22 embryonated egg passages and 15 canine kidney cell culture passages. Drago Ikić initiated subsequent passages in human diploid cell culture WI-38 including three plaquings (in the 9th, 11th and 13th passages); in each, large plaques were selected. The vaccine strain obtained at the end of this procedure represented a homogenous, genetically stable population of virus particles originating from one plaque. In 1967, passage no. 22 on human diploid cells was selected as master seed of Edmonston-Zagreb strain (13). From 1984 the working seed lots have been produced in homologous cell substrate, MRC-5 cells. Several studies



**Figure 3.** The participants of the 3<sup>rd</sup> International meeting of biological standardization hosted by Drago Ikić (third on the right in the first row) in 1957 in Opatija under the auspices of ISM.

have compared the effect of Edmonston-Zagreb and other globally exploited measles vaccine strain by subcutaneous injection at the same dose and age, and found that Edmonston-Zagreb vaccine gave superior seroconversion rates.

The era of discovery continued with adaptation of rubella virus RA 27/3 to HDCS. One feature of the human diploid fibroblast vaccine that differed from the others was the adaptation of the strain to growth at 30°C. The idea behind this was to rapidly induce attenuation, on the basis of previous experience with attenuated poliovirus strains, following the work of others that showed that replication at low temperatures selected against virulence. By the 25th passage in a human diploid cell strain, RA 27/3 clearly became attenuated for humans. All the live attenuated vaccines except RA 27/3, the human diploid cell vaccine, were licensed in 1969 and 1970 in the United States. At the same time, due to Drago Ikić and his collaborators at the Institute of Immunology, RA 27/3 HDCS was licensed in Europe (former Yugoslavia, France). The reason for this difference is of historical interest (14). At the time, there was a prejudice in the American regulatory agency against a human cell strain on the grounds that it might contain some hypothetical contaminating agent. Irrationally, primary cultures from animals were thought to be preferable. But, over the years, human diploid cell strains have become the reference standard for fully characterized cells free of contaminants.

### INTERNATIONALIZATION THROUGH SCIENTIFIC SOCIETIES AND BIOLOGICAL STANDARDIZATION

By the beginning of the twentieth century microbiology went through a phase of rapid proliferation and specialization. Productive research was no longer confined to France, Germany and Britain; first-rate laboratories appeared in the United States, Japan, Russia, Chile, Hungary and many developing countries including former Yugoslavia. As a discipline, microbiology encouraged international collaboration not only through individual efforts but also through scientific societies. The scientific societies concentrated considerable groups of scientists, performed experiments and investigations impossible to individual effort, encouraged individual scientists and gave them both opportunity and leisure for scientific work. They became centers of scientific information, published scientific books and periodicals, propagated periodically scientific discoveries, and thus co-ordinated the scientific efforts of various progressive European countries.

The most prominent society was established as the International Society for Microbiology (ISM) in 1927 at the Pasteur Institute of Paris (the Belgian Nobel Laureate Jules Bordet was elected as the first president). During its long existence, the association has undergone four name changes and eight major reorganizations. Finally in 1980 it grew in to the International Union of

Microbiological Societies (IUMS), an international non-governmental organization which supports the study of microbiological sciences worldwide. It maintains contact with other international organizations such as the UN, UNESCO, WHO and the International Union of Biological Science (IUBS).

Under the auspices of ISM, Drago Ikić hosted in 1957, 1959, and 1960 three symposia in Opatija on biological standardization, immunology and biological standardization, respectively (Figure 3). The meetings themselves brought together scientists of differing training, occupation, and concerns, thereby expanding the views and enhancing the productivity of researchers. Presentations at meetings provide a means for scientists to learn of the most recent discoveries, to gain an overview of the field and to put their own work in a broader scientific perspective. Drago Ikić had valuable presentations at the meetings and was the editor of the proceedings. The proceedings have done much to stimulate cooperation in research, helping scientists and scientific societies to keep in touch with each other.

The international character placed an additional duty on the ISM standardization. The meeting in 1960 was critical in forming a consensus on the core conceptual tools among researchers working on biological standardization, i.e. the standardization of drugs or biological products that cannot be chemically analyzed by studying their pharmacologic action on animals. This international meeting was organized to reach an agreement on a common nomenclature and principles. Drago Ikić's presentation on biological standards encompassed establishment, criteria, stability, present state, requirements, difficulties, improvements, and proposals. He concluded that in the elaboration of concrete proposals for the promotion of biological standardization, the following interests should be borne in mind: promotion of vaccine production process, final vaccine control, development of scientific work in the field of vaccines and interest of the vaccinated population (15).

## FOUNDATION OF WHO COLLABORATING LABORATORY/CENTRE AT THE INSTITUTE OF IMMUNOLOGY, ZAGREB

International health organizations traditionally attempt to standardize procedures for medical microbiological products. The first biological standard in the world, a diphtheria antitoxin serum reference standard, was prepared by Paul Ehrlich in 1897. A whole range of modern remedies (antitoxins, hormones, vitamins, and certain drugs) required that their strength be biologically measured against a standard preparation.

WHO has played a key role for over 50 years in establishing the WHO Biological Reference Materials necessary to standardize biological materials, as well as in developing WHO guidelines and recommendations on the production and control of biological products and technologies. These norms and standards, based on scien-

tific consensus achieved through international consultations, assist WHO Member States in ensuring the quality and safety of biological medicines and related in vitro biological diagnostic tests worldwide. The Organization accomplishes this work through its biological programme, the WHO Collaborating Centers, and the WHO Expert Committee on Biological Standardization (ECBS). This also involves close collaboration with the international scientific and professional communities, regional and national regulatory authorities, manufacturers and expert laboratories worldwide.

By definition (16) »WHO collaborating centre forms part of an inter-institutional collaborative network set up by WHO in support of its programme at the country, intercountry, regional, interregional and global levels. In line with the present WHO policy and strategy of technical cooperation, a WHO collaborating centre must also participate in the strengthening of country resources, in terms of information, services, research and training, in support of national health development...An institution is designated initially for a term of four years; the designation may be renewed...«

Between 1971 and 1997, two WHO Collaborating Centers were active at the Institute of Immunology (Table 1.):

**TABLE 1**

WHO Collaborating Centers in Institute of Immunology.

Period	Title
1971–1997	Biological standardization: Research and reference services (Drago Ikić, Head and Principal Investigator) ( <i>cont.</i> as WHO Collaborating Center for Research and Reference Services for Immunobiological Biological products, dr. Ivanka Čanadija, Principal Investigator)
1973–1995	Reference Centre for References and Research on Bacterial Vaccines and Immunization programmes (Drago Ikić, Head and Principal Investigator)

Reference materials are required to standardize potency, purity, and identity measurements for complex biological materials. For more than two decades Biological Reference Materials from the Institute of Immunology provided a global standard against which experimental values were compared and expressed, allowing direct comparisons between products and measurements across different methodologies and assays in use. The experts from the Institute of Immunology served on the Panel for the Biological Standardization of the WHO from 1957 to 1982. Also, many of them have been assigned as WHO consultants. Headed by Drago Ikić, the Institute organized WHO Interregional Courses for Biological Standardization in 1967, 1968, 1973 and 1976 to support scientific exchange between developed and less-developed countries.

TABLE 2

Year of licensure of selected childhood vaccines.

Vaccine	Year of first US licensure (17)	Year of first Croatian licensure (national producer)
Tetanus toxoid	1943	1954
Inactivated influenza	1945 Trivalent	1970 monovalent type A 1973 bivalent type A+B
Tetanus and diphtheria toxoids	1953 for children aged >7 yrs; 1970 for children aged <7 yrs	1955
Inactivated polio	1955	1959
Oral polio	1963	1961 Koprowski type I and III 1962 Koprowski type II 1968 Koprowski HDCS
Diphtheria–tetanus–pertussis	1970	1960
Diphtheria–tetanus–acellular pertussis	1991	
Measles–mumps–rubella	1963 (measles CEF); 1967 (mumps); 1969 (rubella); 1971 (measles-mumps-rubella combined); 1979 (rubella RA27/3 HDCS)	1967 (measles CEF); 1968 (measles HDCS); 1970 (rubella RA27/3 HDCS); 1972 (mumps); 1974 (measles–mumps–rubella combined)
Hepatitis B	1981 (plasma derived); 1986 (recombinant)	
<i>Haemophilus influenzae</i> type b conjugate	1987 for children aged ≥18 mos; 1990 for infants	
Live attenuated influenza	2003	1968
Meningococcal conjugate	2005	1978 (group A+C)

## VACCINE PREVENTABLE DISEASES, IMMUNIZATIONS AND YEAR OF FIRST LICENSURE 1961-2011

Finally, approval of a vaccine by national regulatory authority for use in the practice based on the results of randomized clinical trials of the vaccine's protective efficacy is ultimate goal for vaccine inventor and scientist. During his active work in vaccine development, manufacture and biological standardization, Drago Ikić and the Institute of Immunology achieved substantial number of licensures. Understanding has been enhanced of diseases now prevented by vaccines, many new vaccines have been introduced and the occurrence of most of these diseases has been dramatically reduced. Moreover, due to close scientific ties with international community, Croatian public health was competitive to the highly developed countries. Table 2 illustrates the comparison between licensure of selected vaccines in the U.S. and Croatia. The year of first Croatian licensure does not correlate necessarily to the introduction of vaccine in the national immunization program.

## NOT MENTIONED ABOVE

From this short text, many accomplishments of Drago Ikić were omitted intentionally. Some very successful

ones are history now. Among them are: vaccination campaign for smallpox eradication (an impressive amount of 30,000,000 doses of smallpox vaccine, Berna-Zagreb vaccine strain, were produced at the Institute of Immunology between 1945 and 1969 for primary vaccination and revaccination of children); design and production of live influenza vaccine in 1962 and field trials in 1964 and 1968 when influenza appeared in Croatia in an epidemic form; research and development of diverse combined vaccines, and cost-effectiveness and cost-benefit analysis of immunization programmes. In 1976 fractionated antigens were developed from whooping cough component (pertussis) of DTP vaccine but research on their potency, stability and reactogenicity was interrupted after Drago Ikić retirement.

At last, but not the least, since foundation of the Institute of Immunology Drago Ikić promoted due to experience with animal antitoxins, the production of medicines derived from human blood and plasma. Normal human immunoglobulin prepared from pooled donations and used for the prevention (or postexposure prophylaxis) of susceptible contacts against common pathogens was licensed in 1962. In 1967, following the analysis of transfusiology development in Croatia and the production of specific immunoglobulins obtained from human plasma, especially of anti-tetanus and anti

Rh0 (D) immunoglobulin, along with already present cryoprecipitate, normal gamma globulin and albumin, the research began of fractionation of human plasma. Licensure of antilymphocyte globulin followed in 1972.

The production and clinical use of human leukocyte interferon (IFN) is a domain where Drago Ikić has been active from the very beginning. Clinical use of interferon worldwide started in 1968. Since 1972, when human leukocyte interferon was licensed in Croatia, until 1975 five hundred and forty patients with viral and malignant diseases underwent interferon treatment. At the same time mode of its action was studied, many scientific papers were published and three international symposia were organized. His interest in interferon is still vivid and hopefully Drago Ikić's research of these immune regulation molecules will draw our attention for many years from now.

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