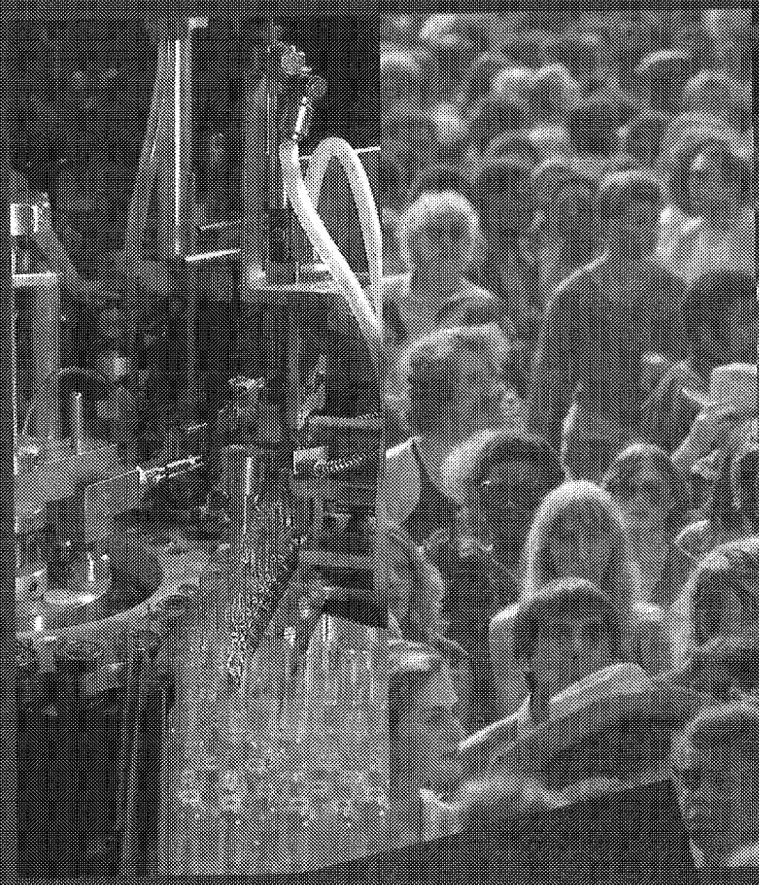
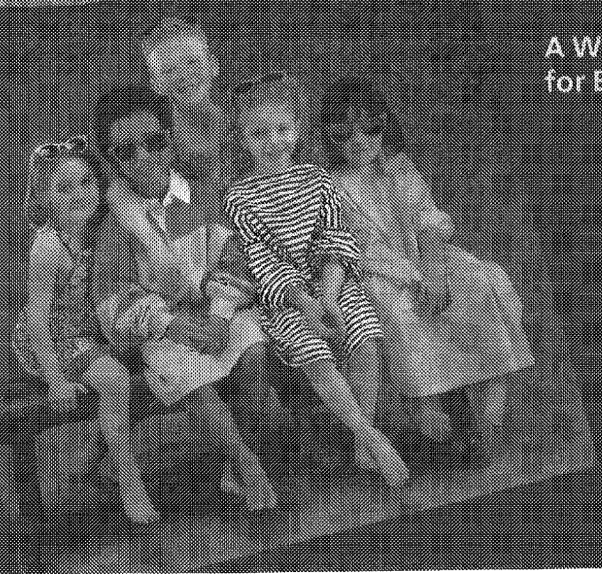
NIBSC

National Institute for Biological Standards and Control

Annual Report 1991–92



A WHO International Laboratory for Biological Standards



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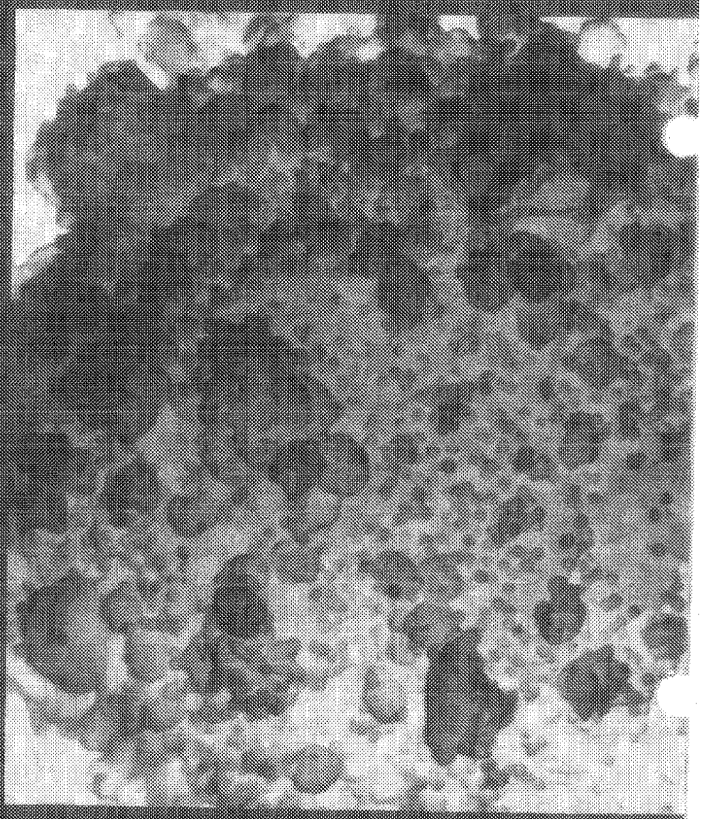
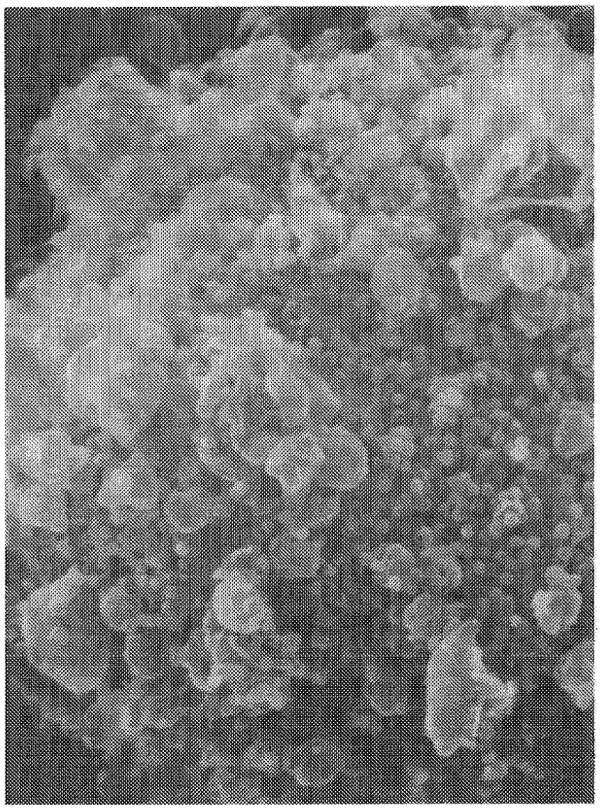


NIBSC

National Institute for Biological Standards and Control

Assuring the quality of biological medicines



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NIBSC's Aim

The aim of NIBSC is to safeguard and enhance public health through the standardisation and control of biological substances used in medicine.

What are Biologicals?

Biologicals are substances used in medicine whose purity or potency cannot be adequately tested by chemical means.

NIBSC Status

NIBSC was formed in 1972 and is managed by the National Biological Standards Board (NBSB). NIBSC and its Board have operated as a Non-Departmental Public Body of the Department of Health since 1975 with functions specified in the NBSB Functions Order of 1976 as called for under the National Biological Standards Act of 1975.

TITLE PAGE
Electron micrograph showing the HIV virus on the surface of infected cells magnified 20,000 times. NIBSC is building a scientific capability for the future control and standardisation of HIV/AIDS vaccines.
(See page 20)

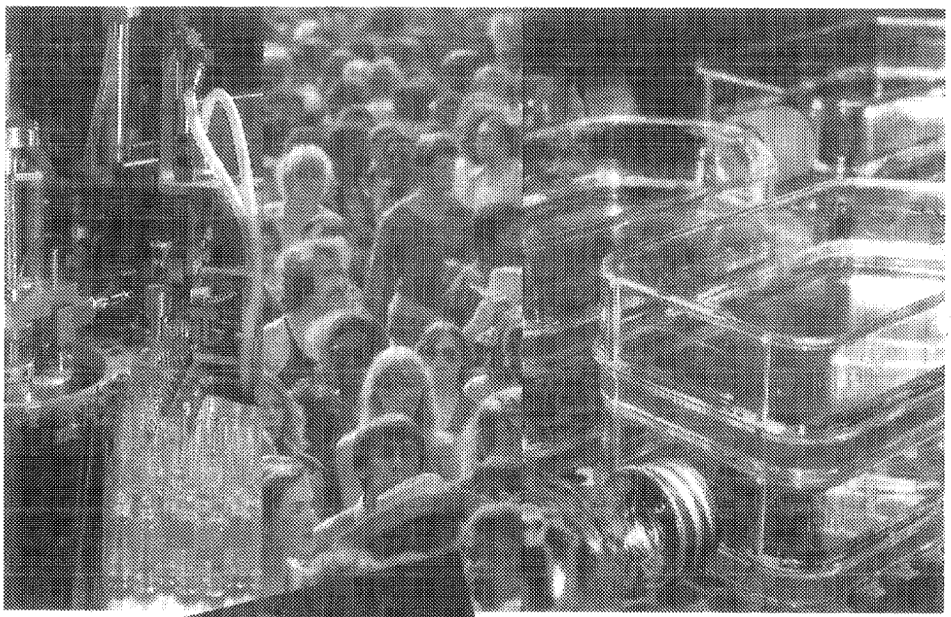
COVER PLATE
Clockwise from left.

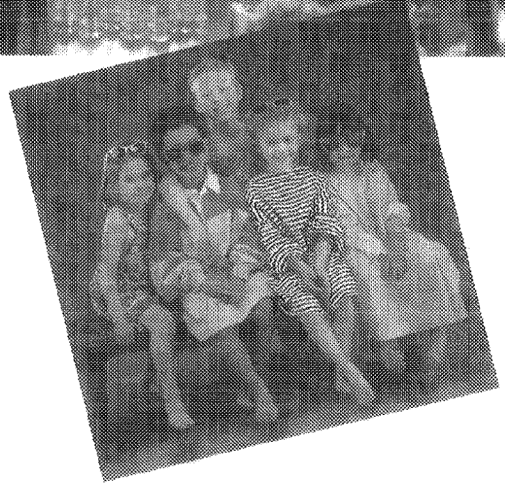
Preparation of ampoules of International Biological Standards, produced and distributed on behalf of the World Health Organization. This work is carried out to exacting standards of accuracy.

The International Biological Standards produced at NIBSC are widely used as the ultimate measures for quality in the biological medicines administered to all age groups worldwide.
(The Telegraph Colour Library)

Studies of virus vaccines in cell culture vessels under carefully controlled conditions at NIBSC, to facilitate testing of vaccine purity and potency.

7,815,000 vaccinations were given to children under 16 in the UK in 1990/91 - each of these vaccines was from a batch tested by NIBSC for safety and efficacy.
(The Telegraph Colour Library)





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Chairman's statement

This report summarises the work of the National Institute for Biological Standards and Control (NIBSC) in 1991/92 and highlights a number of the Institute's recent scientific achievements.

Through its function as a World Health Organization International Laboratory for Biological Standards, NIBSC takes a leading role in providing reference standards for the potency, quality and purity of biological medicines used worldwide in the prevention, diagnosis and treatment of human disease. Literally, NIBSC 'sets the standard' against which preparations of biological medicines are measured. In 1991/92, more than a quarter of NIBSC expenditure went on biological standardisation.

The development of a new standard necessitates a detailed study of a biological substance and of appropriate ways of measuring its activity. Knowledge so gained is invaluable for developing reliable and cost-effective ways of monitoring and controlling the quality of biological medicines, both those on the market and those under development. More than half of NIBSC expenditure in 1991/92 was spent in support of the activities of the Medicines Control Agency, the UK's licensing authority for medicines. Increasingly, these matters are being handled on a European basis, and the Institute's scientists are playing a leading role in the development of the technical issues involved.

The number of biologicals under development continues to increase so that they now constitute the majority of innovative medicines. Their diversity and complexity is also increasing, as novel techniques of biotechnology are transferred from the research laboratory to the production line. This has stimulated a pressing need for new approaches to standardisation and control to complement traditional bioassay procedures.

Recent advances in molecular biology and in the structural chemistry of complex molecules hold great promise, and NIBSC is at the forefront of applying them to the standardisation and control of a wide range of biological substances.

The main purpose of NIBSC, and its major output, is service work in biological standardisation and control on behalf of the UK and international authorities and in support of public health and safety. But throughout NIBSC, this service work is underpinned by high quality research and development. Much of the routine control methodology currently used at NIBSC is based principally on R & D carried out in-house; such development work must continue if tomorrow's problems are to be overcome.

At the time of writing, the Department of Health has just completed a review of NIBSC's role and functioning. The report of the review refers to NIBSC's high reputation among the scientific community and the pharmaceutical industry in Britain and overseas. One outcome of the review is that in future NIBSC's funding will be channelled through a series of agreements with various government bodies and agencies. The review also underlined the central role of the Institute's research and development work on which our ability to keep up with this exciting area of medical science must depend.

The members of the Board pay tribute to all the staff of NIBSC, whose collective skill, dedication and hard work is recorded in this report.

N J B EVANS
Chairman
National Biological Standards Board

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Director's overview

The Institute's programme of work on the standardisation and control of biological medicines is made possible by NIBSC's unique approach to development and research. In 1991/92, high priority was given to the application of advanced biotechnology and instrumentation in the Institute's work and this report describes a number of successful applications in which NIBSC played a pioneering role.

NIBSC was closely involved in technical discussions with various EC groups on the new drug licensing arrangements within the Community. NIBSC's activities on the standardisation and control of biological medicines within Europe are closely co-ordinated with those of the UK Medicines Control Agency. NIBSC has had a key role in the preparation of EC technical guidelines for the manufacture and control testing of different groups of biologicals, as part of the work of the EC's Working Party on Biotechnology and Pharmacy.

Control testing of a wide range of licensed biological products accounts for a major proportion of NIBSC's work. During 1991/92 some 600 licensed products were subject to batch testing, a 19% increase over the previous year, and some 1,991 individual batches of biological products were monitored.

NIBSC's control work is important for the success of national public health programmes. Each of the 7.5 million doses of vaccine used in the national childhood immunisation programme during the year was from a batch subject to control testing at NIBSC. The Government's new targets for increased immunisation coverage and the development of new vaccines will give added importance to NIBSC's control activities.

The Institute was honoured to receive a visit from the Director General of the World Health Organization (WHO), Dr Hiroshi Nakajima, early in 1992. NIBSC has a major role as a WHO International Laboratory for Biological Standards. NIBSC also operates as a WHO Collaborating Centre on Viral Vaccines and for AIDS, as well as working closely with WHO in several other fields.

During the year, 6 new WHO International Standards for biological substances were established and over 40 standards and other reference materials were developed. These activities involved NIBSC in a broad programme of international collaboration.

NIBSC maintains an extensive stock of International Standards and other materials which are distributed worldwide and contribute significantly to the quality of biologicals. There has been a general increase in demand for these materials over the past five years reflecting the heightened industrial interest in biological medicines.

To ensure the effective use of the Institute's resources considerable effort has been devoted to the development of improved management systems during 1991/92.

This report summarises our control and standardisation results and features selected scientific highlights. The latter illustrate the range and nature of the Institute's scientific work and the contribution it makes to securing high standards of quality, efficacy and safety of biological medicines. These achievements reflect the teamwork and high level of commitment shown by the staff of the Institute.

A list of the scientific papers written by NIBSC staff and published during the year is available from our librarian on request.

G C SCHILD
Director

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Control testing

NIBSC's role

In the UK, NIBSC assists in ensuring high standards of quality and reliability for biological medicines through a continuing series of interactions with the Medicines Control Agency (MCA) of the Department of Health and with pharmaceutical manufacturers.

This process starts during the product development phase when manufacturers are encouraged to discuss with MCA and NIBSC scientists proposed procedures for manufacture and quality control of products. One forum for this dialogue is provided by NIBSC's Advisory Group on Biotechnology.

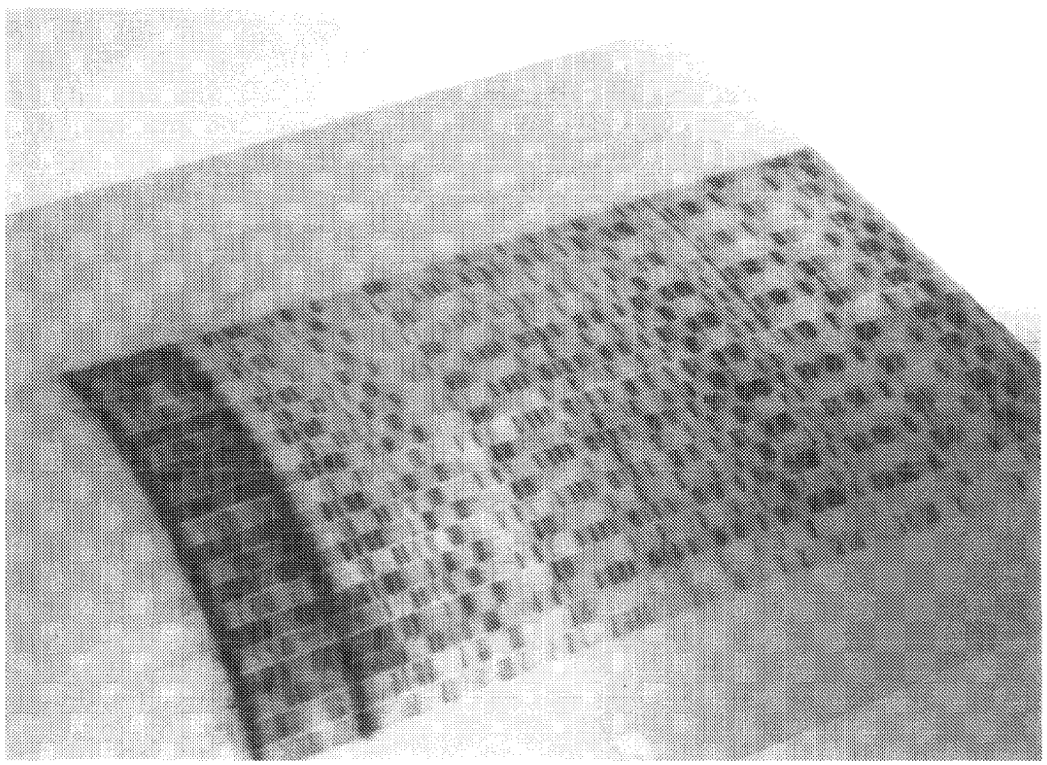
The processes of product monitoring also involve continuing exchanges of information with the manufacturers on technical aspects, such as assay syatems and the appropriate use of standards.

A pharmaceutical company seeking to market a biological medicine in the UK is required to submit a licence application to the MCA. Licence applications are examined in depth by the MCA who are advised by the Biologicals Sub-Committee of the Committee on Safety of Medicines, an independent body. NIBSC is represented on this Sub-Committee.

The MCA issues many licences on the basis that samples of the product are submitted to NIBSC for testing; this is done through a system of batch examination orders.

The MCA frequently seeks the advice of NIBSC scientists on technical aspects of applications. The advice given includes comments on the nature and suitability of source materials, the manufacturing strategy, in-process control and final product specification and product. Another important issue is the appropriate use of standard preparations in the manufacturer's quality control processes.

Licensing of medicines in member states of the European Community is now subject to the emerging EC procedures for European licence applications. NIBSC is closely involved with MCA in the development of these procedures at the European level to ensure that the highest standards of safety and efficacy of biological medicines available in the UK are maintained.



DNA and RNA sequences are determined by modern methods involving gel electrophoresis, as illustrated here. The technique is used throughout NIBSC by scientists studying the genetic make-up of bacteria and viruses. Knowledge of the molecular biology of such micro-organisms allows NIBSC to monitor the purity and potency of vaccines to the highest scientific standards.

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Table 1 Activities relating to control of biologicals, 1991/92

NIBSC department	*Control testing*			*No. of examinations of license applications*
	No. of batches of products examined	*No. of batches considered unacceptable or withdrawn by manufacturer*	*No. of other interventions by NIBSC*	
Bacteriology	357	4	41	20
Chemistry	109	1	13	23
Endocrinology	251	0	50	8
Haematology	665	7	60	8
Immunobiology	240	1	35	21
Virology	369	4	50	17
All departments	**1,991**	**17**	**249**	**97**

Examination of licence applications and control specifications for biological products at NIBSC. A product licence application prepared by a manufacturer is submitted initially to the Medicines Control Agency (MCA) of the Department of Health. NIBSC advises MCA on scientific and technical issues relating to the licensing of biological medicines.

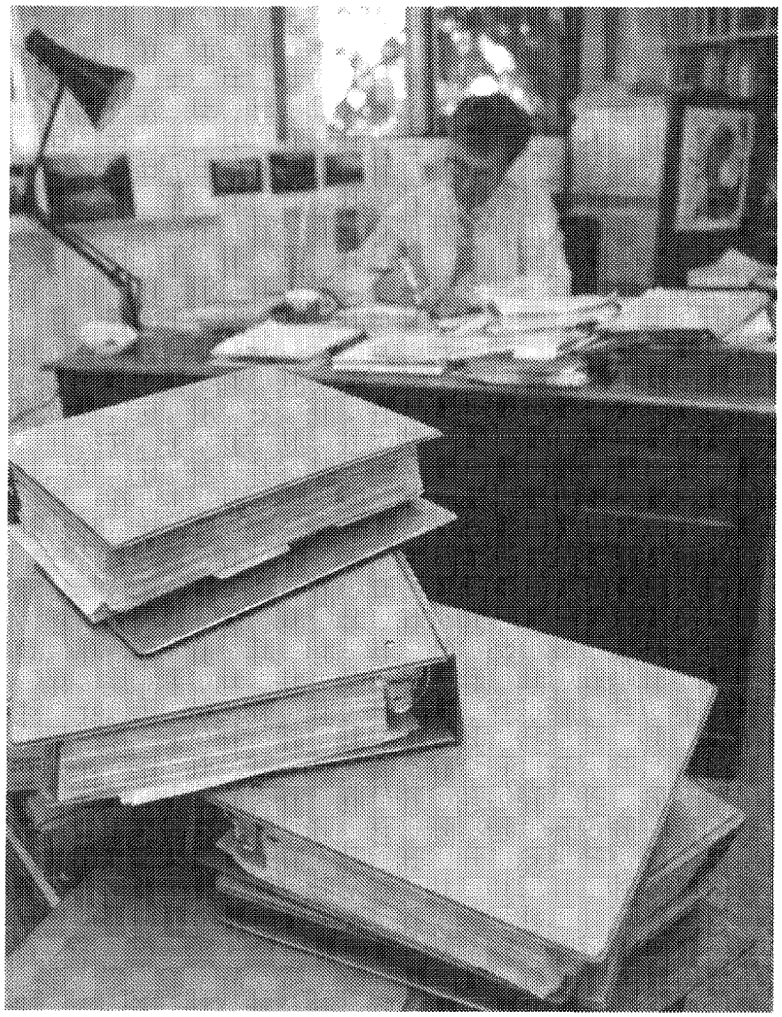
Control results

The number of products to which batch examination were applicable increased from 580 at the beginning of 1991 to over 600 in March 1992. The volume of control testing and advisory work related to licence applications is summarised in Table 1. A total of 1,991 batches of products intended for use in the UK were evaluated in 1991/92 (1,477 in 1990/91).

Of the total number of batches evaluated, 17 (0.85%) were considered unacceptable or were withdrawn by the manufacturers (compared to 30 (1.5%) in 1990/91).

NIBSC gratefully acknowledges the helpful co-operation of the manufacturers of licensed products in the provision of materials and information.

The following were the key features of NIBSC's control work during 1991/92:



Bacterial Vaccines

The control of bacterial vaccines used in childhood vaccination programmes was a major aspect of the work of the Bacteriology Division. Some 200 batches of BCG vaccine, 60 batches of diphtheria/pertussis/tetanus (DPT) vaccine and 41 batches of tetanus vaccine were evaluated.

The effectiveness of national immunisation programmes, and the safety of vaccines used in them, is aided by NIBSC's work. In 1990/91 in England

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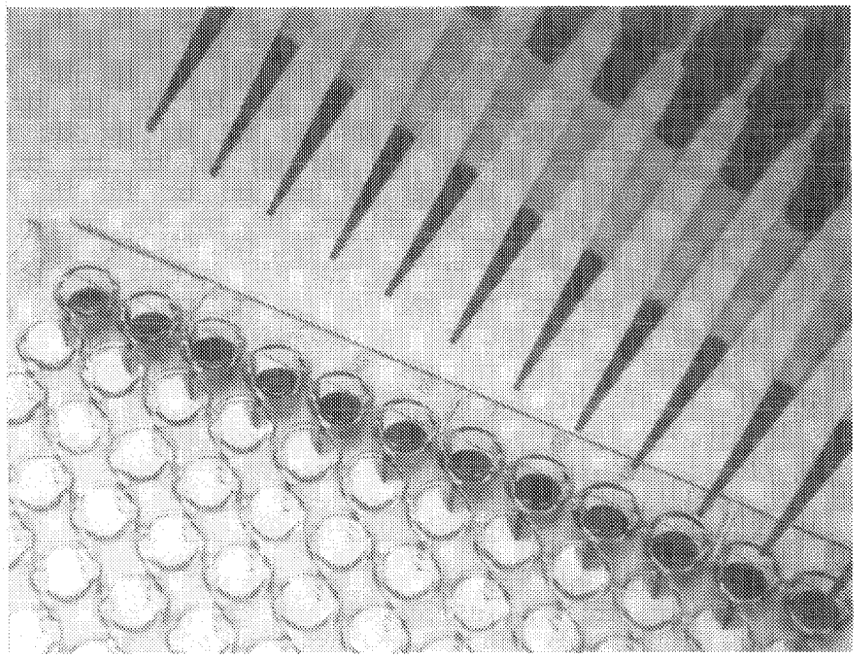

Pipetting techniques for modern assays require microlitre (one millionth of a litre) volumes to be measured. The apparatus shown permits such volumes to be dispensed speedily and safely.

197,000 BCG vaccinations were administered; in the same period, 539,000 children were vaccinated against diphtheria, 1,070,000 against tetanus and 581,000 against pertussis. Each vial of vaccine used in these programmes came from a batch tested by NIBSC.

Several batches of vaccine against the A & C serogroups of *Neisseria meningitidis*, which is a major cause of childhood meningitis, were assessed.

As meningitis vaccines are developed, NIBSC is developing independent tests to ensure vaccine safety and effectiveness. In 1991, 2,744 cases of meningitis were reported in England and Wales.

Viral Vaccines

The Virology Division assessed 143 batches of oral polio vaccine and 109 batches of influenza vaccine.

Some 900,000 primary courses of childhood vaccination against poliomyelitis were completed in England in 1990/91. Vaccination against polio is included in the national target of 95% immunisation coverage by 1995; attaining this target will depend on the reliability and safety of vaccines, which NIBSC helps assure.

Thirteen batches of combined measles, mumps, rubella (MMR) vaccine were also evaluated against agreed licence specifications.

In 1990/91, 1,060,000 children were vaccinated using MMR vaccine.

Following substantial development work at NIBSC on standardisation and control of hepatitis A, the first batches of commercial hepatitis A vaccine were assessed during the year.

This recently licensed product represents an important milestone in the prevention of hepatitis. Its use should reduce significantly the incidence of viral hepatitis in the UK (9,000 cases in 1990).

Cytokines and Hormones

The Immunobiology Division evaluated 58 batches of interferons intended for therapeutic use. Fourteen batches of a newly licensed cytokine, granulocyte macrophage-colony stimulating factor (GM-CSF) were also tested.

The interferons examined are used primarily in the treatment of cancer and viral infection. The clinical applications of GM-CSF include bone marrow transplant therapy, treatment of leukaemia and the experimental treatment of HIV infection and AIDS.

The Endocrinology Division tested 169 batches of 30 different insulin products.

It is estimated that around 0.75 million people in the UK suffer from diabetes, and that 4 to 5% of total health care expenditure is spent on the care of diabetics. Internationally, more than £200 million was spent on human insulin products in 1990.

The Endocrinology Division also evaluated 10 batches of erythropoietin, a recombinant DNA-derived hormone.

Erythropoeitin has been licensed for the treatment of dialysis anaemia in patients with renal disorders, and has had a profound effect on the quality of life in such patients since its initial approval in 1989.

Blood Products and Antithrombotic Agents

The Haematology Division examined 665 batches of blood clotting factors, other blood products, thrombolytic drugs and anticoagulant agents. A wide variety of specialised tests were used in the assessment of these products.

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Thrombolytics and anticoagulants account for around 3.5% of NHS expenditure on medicines, and are used in the treatment and prevention of heart disease and strokes. The latter together accounted for 38% of all deaths in the UK in 1989 and result in 42.5 million lost working days per year. The UK aims for a 30% reduction in deaths under 65 years from these diseases by the year 2000.

Control Findings

The majority of queries arising during product monitoring relate to the biological potency of the product (ie units of biological activity per dose). This is clearly defined in the product licence specification and has direct relevance to a product's effectiveness. For example, a vaccine whose antigen content is lower than specification is unlikely to be fully immunogenic leaving a proportion of recipients unprotected against the disease in question.

When control testing reveals such problems or inconsistencies of data, technical discussions with the manufacturer on assay techniques and on the use of appropriate standard preparations often resolve the difficulty and prevent its recurrence. Some findings also provide the impetus for international collaborative studies which can provide the scientific basis for devising improved testing methods.

One critical aspect of control is the purity of a biological. The licensing of recombinant DNA-derived products requires manufacturers to provide evidence that potentially harmful materials (eg viruses or potentially oncogenic DNA) are not present in the product. Since minute changes to the commercial production process can result in significant changes in the degree of purification achieved, detailed monitoring of purity and consistency of composition of the product is an important aspect of control procedures.

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Biological standards

Strategy of standardisation

The practice of using stable, well-characterised preparations of biological substances as standards against which to assess batches of research materials or manufactured products was developed in the UK over 65 years ago. It is now universally adopted, and remains fundamental to the control of almost all biological medicines whether prepared by conventional means or new biotechnological methods.

The International Biological Standards produced by NIBSC are the 'yardsticks' used to ensure comparability of quantitative measurements of biological activity of individual biological substances. Thus wherever and whenever a biological medicine such as Factor VIII, human growth hormone or a measles vaccine is prepared in the world its quality and potency is assessed directly or indirectly against the appropriate International Standard preparation produced and distributed by NIBSC.

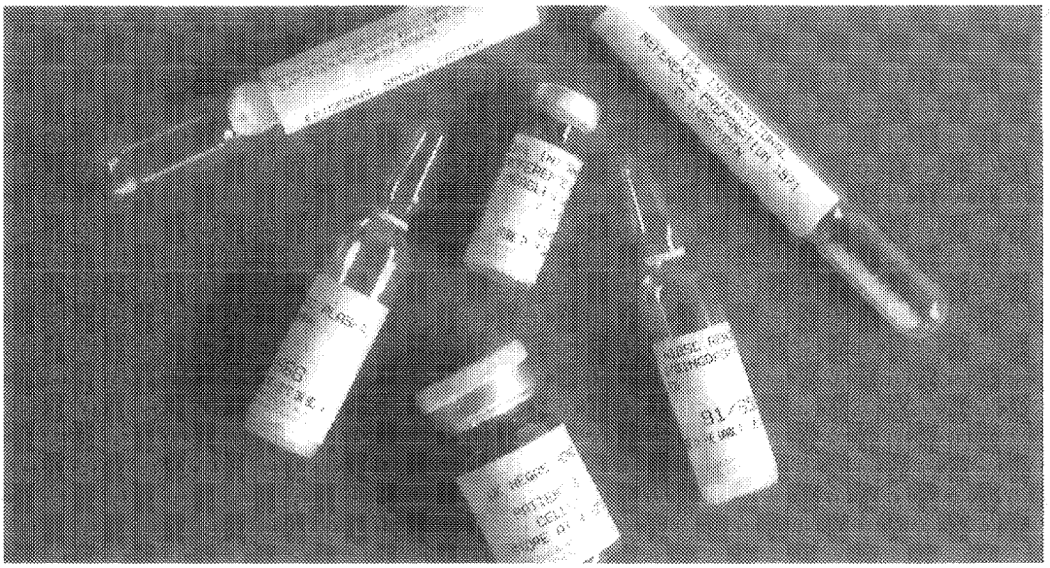
An International Standard is prepared in a homogeneous batch comprising a large number of identical ampoules of the substance constituted in such a way as to stabilise its biological properties. Calibration of a standard is normally in units of biological activity per ampoule, thus ensuring the uniformity of the potency measurements for biological medicines wherever they are made or used.

As the technique of assay of each type of biological substance is unique, each standard requires for its development specialist scientific expertise. To qualify for the designation of International Standard, candidate materials need to meet exacting WHO specifications, including evidence of long-term stability and fine limits for the reproducibility of the content of individual ampoules.

Each candidate standard is subject to an extensive international collaborative study, organised by NIBSC, to provide the scientific basis for the allocation of International Standard status. International Biological Standards are valuable materials and for practical reasons have to be prepared in limited amounts and are used sparingly. These standards are used for the calibration of national and working standards; major users of biological standards are national control laboratories, academic laboratories and commercial manufacturers.

In addition to developing International Standards, NIBSC processes UK Standards for the NHS and the UK Blood Transfusion Service, and prepares reference materials for a range of users, including the EC's Community Bureau of Reference.

NIBSC's Standards Processing Division is equipped with specialist equipment for high quality processing, ampoule filling, freeze-drying and packaging of biological standards.



A selection of biological reference materials processed and distributed by NIBSC. Biological standards produced at NIBSC are required to retain their biological activity over a 20-year period.

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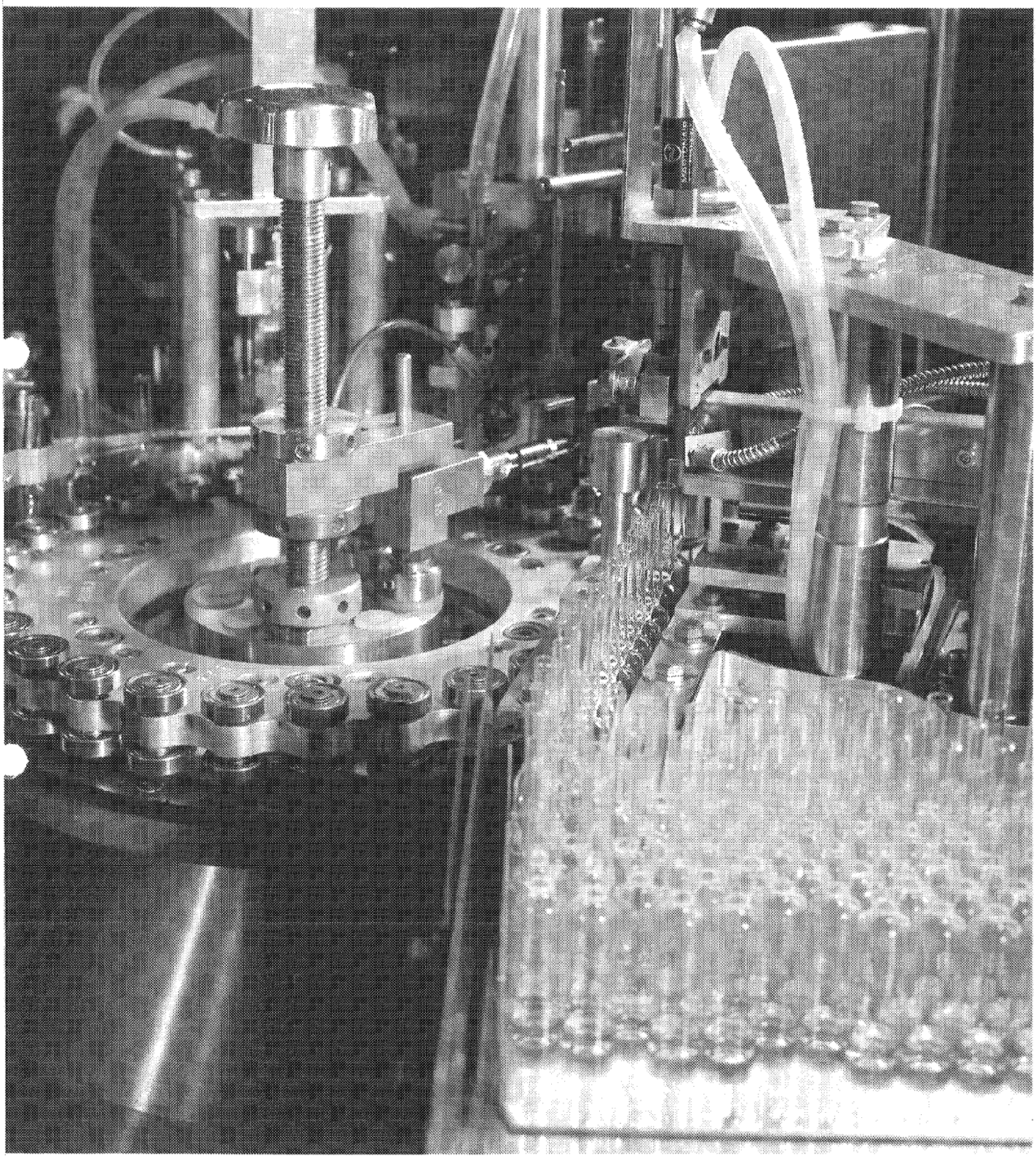
The NIBSC-modified machine used for the accurate filling and sealing of ampoules of biological standards and other preparations. A coefficient of variation of less than 0.25% is obtained on all ampoule filling operations. This standard of accuracy and reproducibility is essential for the preparation of international standards.



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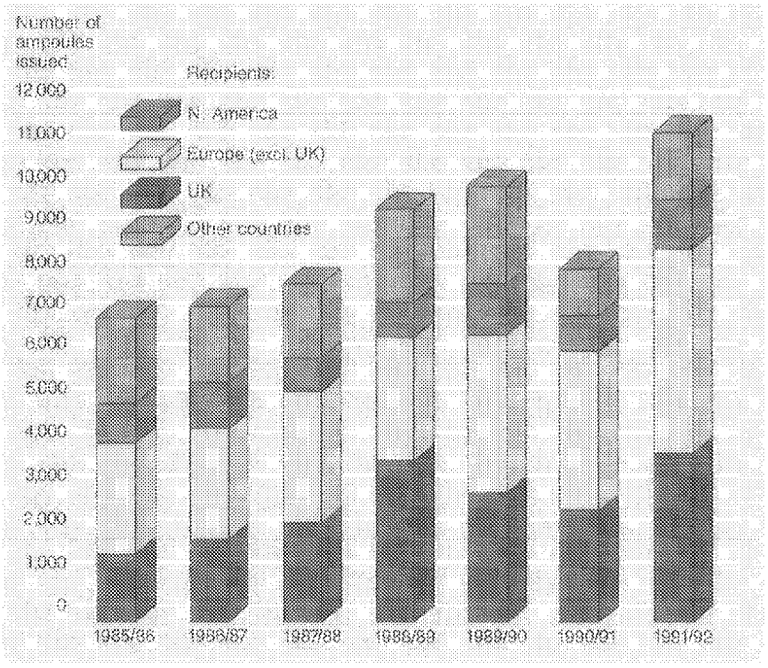

FIGURE 1
Distribution of Standards by NIBSC: WHO International Standards.

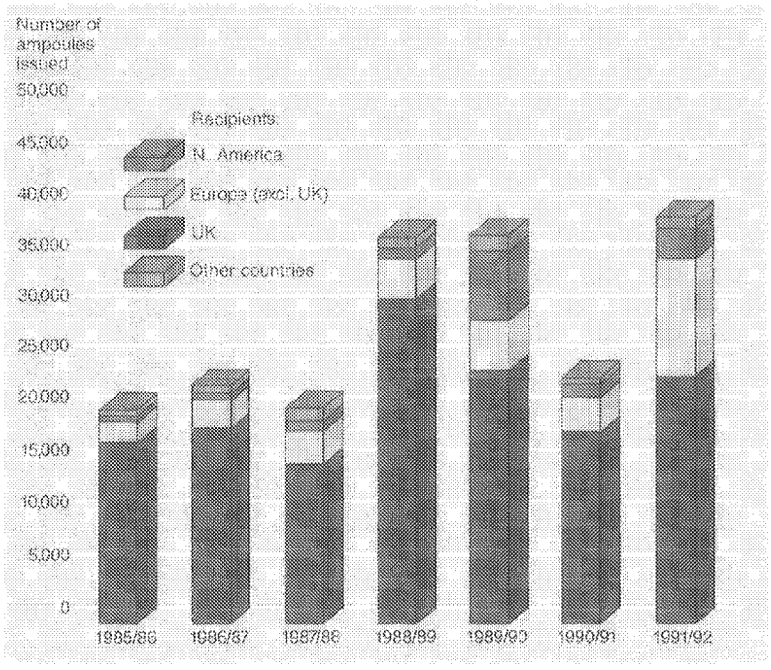

FIGURE 2
Distribution of Standards by NIBSC: Reference preparations other than International Standards.

1991/92 Standards results

International Standards

During the year, NIBSC preparations of the following biologicals were established by WHO as International Standards:

Anti-polio sera (types 1, 2 & 3)
Recombinant Tumour Necrosis Factor alpha (rhTNF-a)
Thyroxine binding globulin
Alpha Thrombin, human
Calcitonin, human
Calcitonin, porcine

All Preparations

Some 100,000 ampoules of candidate International Standards, UK Standards and other reference materials were prepared. These ampoules were prepared in a series of 70 production batches, each comprising a different biological preparation.

Distribution

The number of ampoules of Standards distributed by NIBSC over the past seven years has increased steadily as shown in Figures 1 and 2.

Collaborative Studies

To develop International Standards and other reference materials, NIBSC co-ordinates international collaborative studies, involving around 30 laboratories. During 1991/92, the Institute was involved in the design and analysis of results of some 35 studies of this type.

AIDS Reagents

Through its AIDS Reagent Repository (funded by the Medical Research Council, EC and WHO), NIBSC supplies reference reagents for use in research and development work on AIDS worldwide. The reagents distributed included peptides, monoclonal antibodies, cell lines, DNA clones and biologicals used in PCR procedures and virus strains of HIV-1, HIV-2, SIV and FIV. During 1991/92 there were 3,303 requests for individual reagents from the Repository.

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Scientific highlights

NIBSC's control and standardisation activities are made possible by a programme of research and development for improved techniques. This work is undertaken often in collaboration with other scientific groups in academic or industrial settings. Research and development is targeted at the design and evaluation of new and improved laboratory techniques and ensures that NIBSC has the appropriate scientific and technical information and expertise needed to develop control strategies and standards for future products.

All research activities are kept under close review to ensure both relevance to NIBSC's scientific priorities and high standards of scientific quality. The need for an active research programme is underlined by the current rapid rate of development by industry of novel biological products.

Described below are a number of different aspects of research and development work which illustrate the range of scientific activities at NIBSC and their importance for standardisation and control.

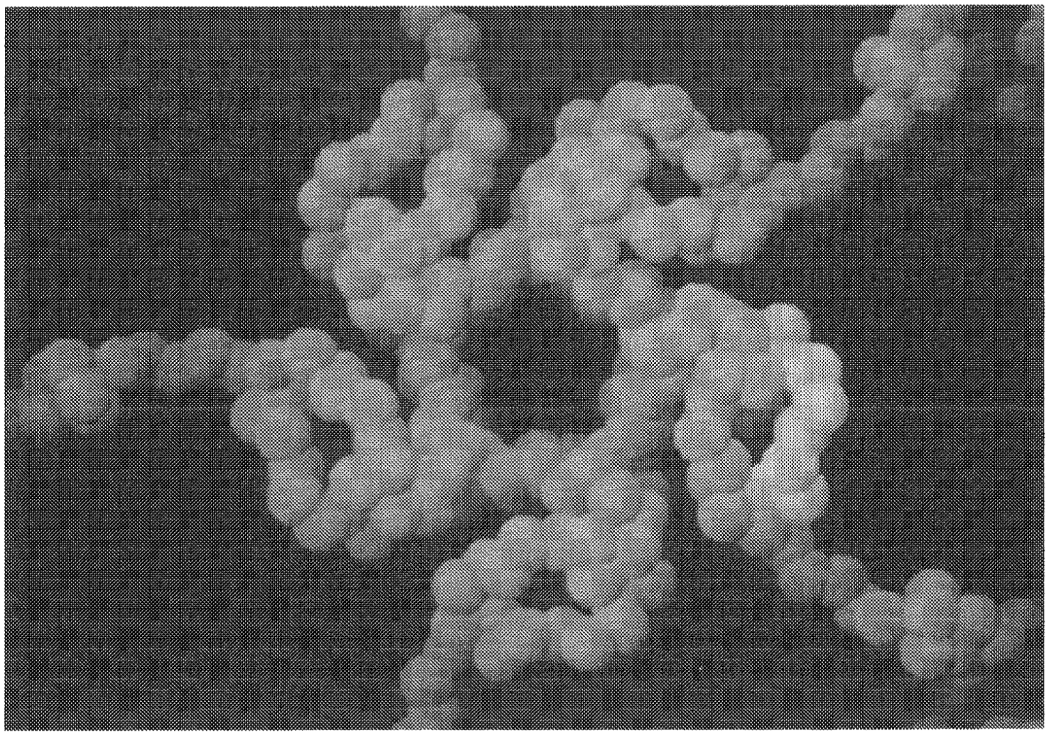
Improving poliovaccine testing

Background

During the 1950s epidemics of poliomyelitis occurred in the UK causing several thousand cases of paralytic disease with a high incidence of mortality. Through the use of vaccines, poliomyelitis is now very rare in developed countries. However, there is a low but real risk of vaccine-associated poliomyelitis, and it follows that all batches of vaccine must be thoroughly tested for safety before use.

Live, Sabin Vaccines

Studies on the molecular biology of live attenuated (ie made less virulent) poliovaccines has led to the precise determination of the molecular basis for the attenuation of Sabin vaccine strains. By enabling the mechanisms of attenuation to become clearly established, this work has allowed the development of tests for the identification of virulent revertant viruses in vaccines. The research has also led to the possibility of constructing, by genetic engineering, derivatives of the Sabin vaccine strains, which are less likely to revert to virulence.



A 3-D computer-generated model of five copies of the protein VP4 found in poliovirus. The protein is buried deep within the virus, and so its possible involvement in the immune response was not expected. The two epitopes recognised by virus-specific cell clones are highlighted. Clarification of the role of VP4 could lead not only to improved control testing of poliovaccine, but also the development of improved vaccines.

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Inactivated Vaccines

Other studies concern the control of inactivated poliovaccines, which remain the basis of poliovaccination in several countries and continue to be used in the UK in individuals in whom live vaccines are contra-indicated. Inactivated vaccines are preparations of poliovirus rendered non-infective by treatment with formalin. Control tests to assess the antigenic potency of vaccine batches are of critical importance; in some countries outbreaks of poliomyelitis have been attributed to the use of subpotent vaccines.

However, in these tests occasional batches have failed to give any response for unknown reasons. So in order to gain a better understanding of the immunological properties of the virus and thus improve potency testing, a NIBSC project has examined the cellular immune responses to poliovirus in murine models.

A major advance in this project has been the preparation of cell clones from murine strains that are poliovirus specific. Surprisingly, a majority of these clones recognise two epitopes of a small capsid protein known as VP4, which is buried in the intact virus (see illustration) - VP4 has not previously been thought to be implicated in the immune response, and its role needs further evaluation.

Such studies enable NIBSC to develop and refine its control tests to ensure the highest standards of purity and potency testing of biological medicines.

Measles/mumps/rubella vaccines

In 1988 measles/mumps/rubella (MMR) vaccines were brought into use in the UK. These vaccines contained the Urabe vaccine strain of mumps. An MMR vaccine containing the Jeryl Lynn strain of mumps was also licensed in the UK. Shortly after the introduction of these vaccines a small number of cases of meningitis associated with vaccination were reported in recipients. While the affected children were hospitalised the course of the disease was benign and no long-term sequelae have occurred.

NIBSC was asked to identify the mumps virus present in cerebro spinal fluids of children with vaccine-associated meningitis. By using gene sequencing methods, NIBSC staff were able to identify unambiguously the virus in each of the 10 vaccine-associated cases examined as the Urabe strain. The Jeryl Lynn strain of mumps virus has not so far been implicated in vaccine-associated meningitis.

Assay of the sera of vaccinees for mumps neutralising antibodies carried out at NIBSC showed that the Urabe and Jeryl Lynn vaccine strains were of comparable immunogenicity. The incidence of adverse reaction to the Urabe strain was initially estimated as one per 100,000 to 230,000 doses distributed, but later studies carried out by the Public Health Laboratory Service (PHLS) suggested that the true incidence of cases requiring hospitalisation was near 1 in 11,000. Purchase of the MMR vaccines containing the Urabe strain was therefore suspended by the Department of Health in September 1992, leaving only the vaccine containing the Jeryl Lynn strain in routine use.

In NIBSC studies on vaccines containing the Jeryl Lynn mumps vaccine strain an unexpected finding has been the presence of two genetically distinct populations of virus in all vaccine preparations so far examined by gene sequence methods. One population is four times more abundant than the other. The significance of this finding in relation to the safety and consistency of the vaccine is under further investigation.

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Improving testing of pertussis vaccines

Infection by *Bordetella pertussis* (the causative agent of whooping cough) remains an important cause of morbidity in infants. The bacteria have also been implicated in respiratory disease among adults. Pertussis infection is controlled effectively by vaccines that consist of intact, inactivated *B. pertussis* bacteria – the so-called 'whole cell' vaccines.

Concern over the possible side-effects of the whole cell vaccine has stimulated extensive efforts to develop new acellular vaccines based on purified antigenic components of the organism rather than whole cells.

NIBSC has been involved in the elucidation of the basic mechanisms of immunity to *B. pertussis*. This has been assessed at NIBSC using an experimental murine model involving respiratory challenge with a *B. pertussis* aerosol (Figure 3). Studies have shown that there is a strong correlation between protection and the development of cellular immune responses involving T cells.

While effective whole cell vaccines stimulate both good antibody and T cell responses, certain acellular vaccines, although producing good antibody responses, stimulate poor T cell responses and give little protection in the experimental model.

Use of such a model may be the key to determining the potency and effectiveness of different acellular pertussis vaccines in the future.

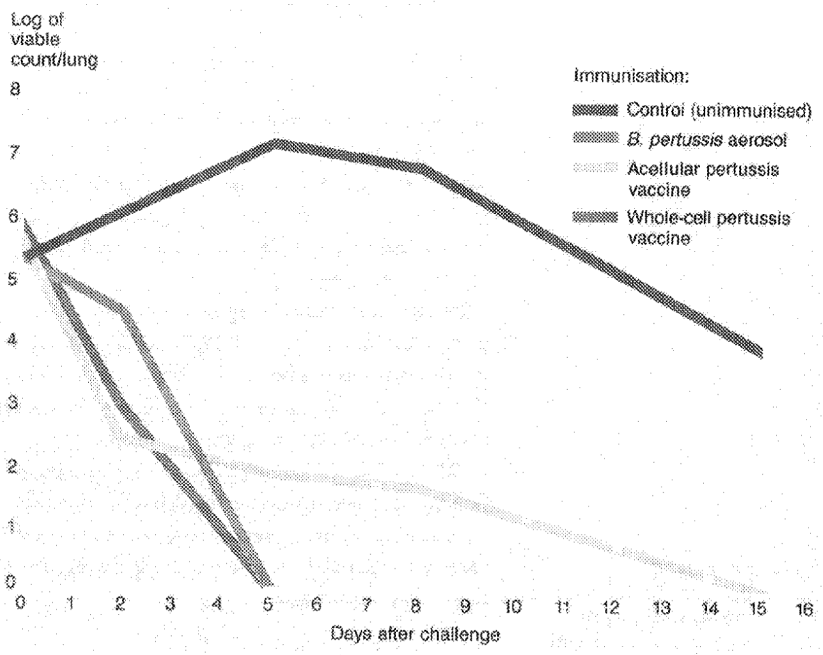
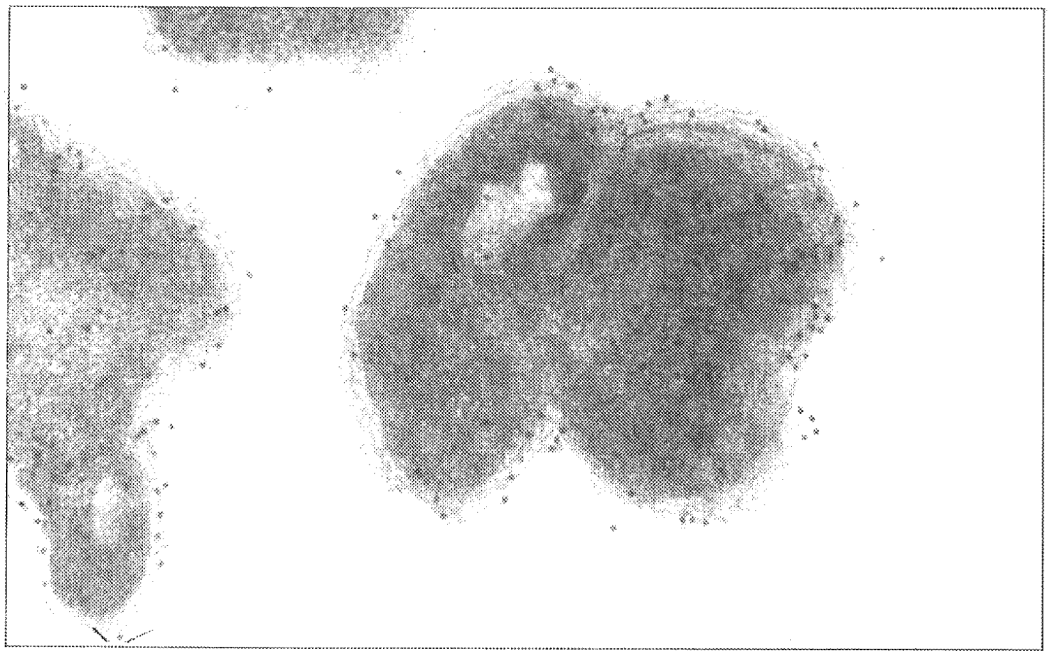


FIGURE 3
Rate of clearance of *B. pertussis* strain W28 from the lungs of different treatment groups following aerosol challenge (murine model). Lung infection is cleared by day 5 in groups immunised by aerosol or by whole-cell vaccine, and much more slowly in the acellular vaccine group.



Cells of meningococcus *(N. meningitidis)* magnified 45,000 times. The location of one of the surface proteins that could be important in future vaccines has been indicated using monospecific antibodies linked to gold, seen here as black dots.

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Assessing meningitis vaccines

Background

The principal causes of bacterial meningitis are *Haemophilus influenzae* serotype b ('Hib'), meningococcus and pneumococcus. Serious disease caused by *H. influenzae* b is uncommon after the age of 5, but the meningococcus (see illustration on page 15) and pneumococcus also affect other age groups.

Disease caused by *H. influenzae* b can be prevented by vaccines. Vaccination against *H. influenzae* b was introduced into the UK infant immunisation schedule in 1992. NIBSC has been involved in pre-licensing examination of candidate Hib conjugate vaccines and has devised appropriate test procedures for batch control. The quality of these vaccines will be monitored closely, particularly because of their novel use in the infant population.

Current Progress

Most of the meningococcal disease in the UK and other developed countries is caused by meningococci of serogroup B. The capsular polysaccharide of this group of bacteria is poorly immunogenic in man and no effective vaccine is currently available. Attempts to develop effective vaccines have centred around the major outer membrane proteins of the bacterium. In 1991/92, at the invitation of the World Health Organization, NIBSC laboratories evaluated two such vaccines which had been developed in Cuba and Norway. These vaccines were found to be complex mixtures of components, including membranous vesicles.

Because of the complexity of such vaccines it is not certain which components are essential for efficacy. To determine this, NIBSC's Bacteriology Division has undertaken a programme of characterization of the meningococcal outer membrane proteins.

Regions of the genes that encode specific outer membrane proteins of meningococcus have been cloned and expressed as fusion proteins in *Escherichia coli* (a bacterium). These have been used to develop highly specific tests that allow the role in immunity of individual vaccine components to be examined. These tests have been refined using PCR technology, and have led to NIBSC developing a new DNA-based technique for identifying strains of meningococcus (see Figure 4). Use of a technique called pulsed field gel electrophoresis has led to a simplified method for characterisation of epidemic clones.

These procedures are proving valuable in studying the epidemiology of meningococcal infection in the UK and abroad and are being applied to vaccine assessment through characterisation of strains isolated from individuals vaccinated in clinical trials.

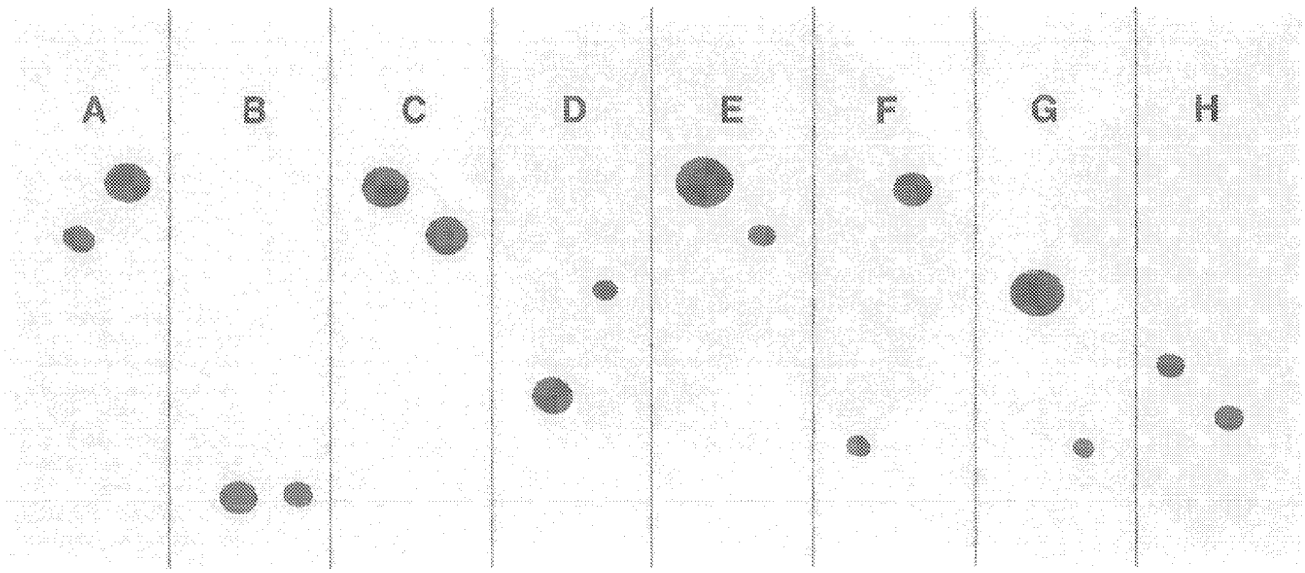

FIGURE 4
Dot-blot hybridisation for the identification of meningococcus strains. This novel DNA-based technique developed by NIBSC allows each of the eight strains of meningococcus (A to H) to be identified by its distinctive pattern. Such techniques of molecular biology are important new tools in the development and standardisation of new vaccines.

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Standards for cytokines

Cytokines in Medicine

Cytokines are protein molecules found in the body that control many of the immune processes at the cellular level and regulate the growth and functioning of blood cells. Cytokines also mediate many of the effects associated with physiological or pathological disorders, eg inflammations or anti-viral responses. In broad terms, cytokines can be thought of as hormones acting at the cellular level.

Although the earliest family of cytokines (the interferons) was recognised 30 years ago, it is only more recently that the great variety of functions, and the clinical therapeutic potential, of cytokines has been recognised.

Cytokines with therapeutic potential can give highly effective treatment of specific disorders.

A number of cytokines have now been licensed for medical use, and cytokines represent an important and rapidly developing part of the pharmaceutical industry's activities.

During the past few years more than 30 cytokine genes have been cloned enabling the production of large amounts of cytokine proteins of high purity.

The cytokines most widely used for human therapy to date are the interferons (used for treatment of eg leukaemia), erythropoeitin (treatment of anaemia associated with renal failure), interleukin 2 (treatment of eg renal cell carcinoma), and the colony-stimulating factors (eg to boost bone-marrow reconstitution).

Cytokines at NIBSC

NIBSC has developed and evaluated several cell-line based bioassays which can be used to determine the potency of cytokines and to detect cytokines in preparations of other biologicals. By using these bioassays in combination with techniques involving neutralising antibodies, individual cytokines can be recognised.

Cytokines may also occur as unwanted contaminants of other biological medicines and could cause adverse effects in recipients. Work at NIBSC has shown that the crude materials used for production of several biologicals can contain appreciable quantities of 'inflammatory' cytokines and processes used to manufacture therapeutic grade products should therefore be designed to remove these undesirable contaminants.

Further studies at NIBSC have been conducted to attempt to understand the mechanism of cytokine action more fully, and to investigate the possibility of modifying signal transduction pathways to produce more sensitive, specific and reliable bioassays.

The preparation and establishment of standards for cytokines is a high priority of NIBSC's Immunobiology Division. So far 25 International Standards of reference preparations for cytokines have been prepared at NIBSC and are available for distribution. The NIBSC programme on cytokine standardisation is conducted in close collaboration with the Center for Biologics Evaluation and Research (part of the US Federal Drug Administration), the National Institute of Allergy and Infectious Diseases and the National Cancer Institute.

NIBSC could not achieve its objectives without the helpful collaboration of the pharmaceutical industry in providing cytokines as candidate standards and participating in extensive collaborative studies.

Analysing molecular structure

Background

Over the past few years developments in physicochemical techniques have made them increasingly valuable in the control and standardisation of biological medicines. While bioassays can show what a biological product *does*, spectroscopic methods can show what it *is* and what impurities it contains.

Spectroscopic data can thus be used to monitor both purity and consistency of manufacture. More detailed information on the relationship between chemical structure and biological activity, which may be critical to future control and standardisation methods, can also be obtained.

For the control of many products, NIBSC's multidisciplinary approach involves the use of complementary techniques of bioassay, molecular biology, protein chemistry and spectroscopy.

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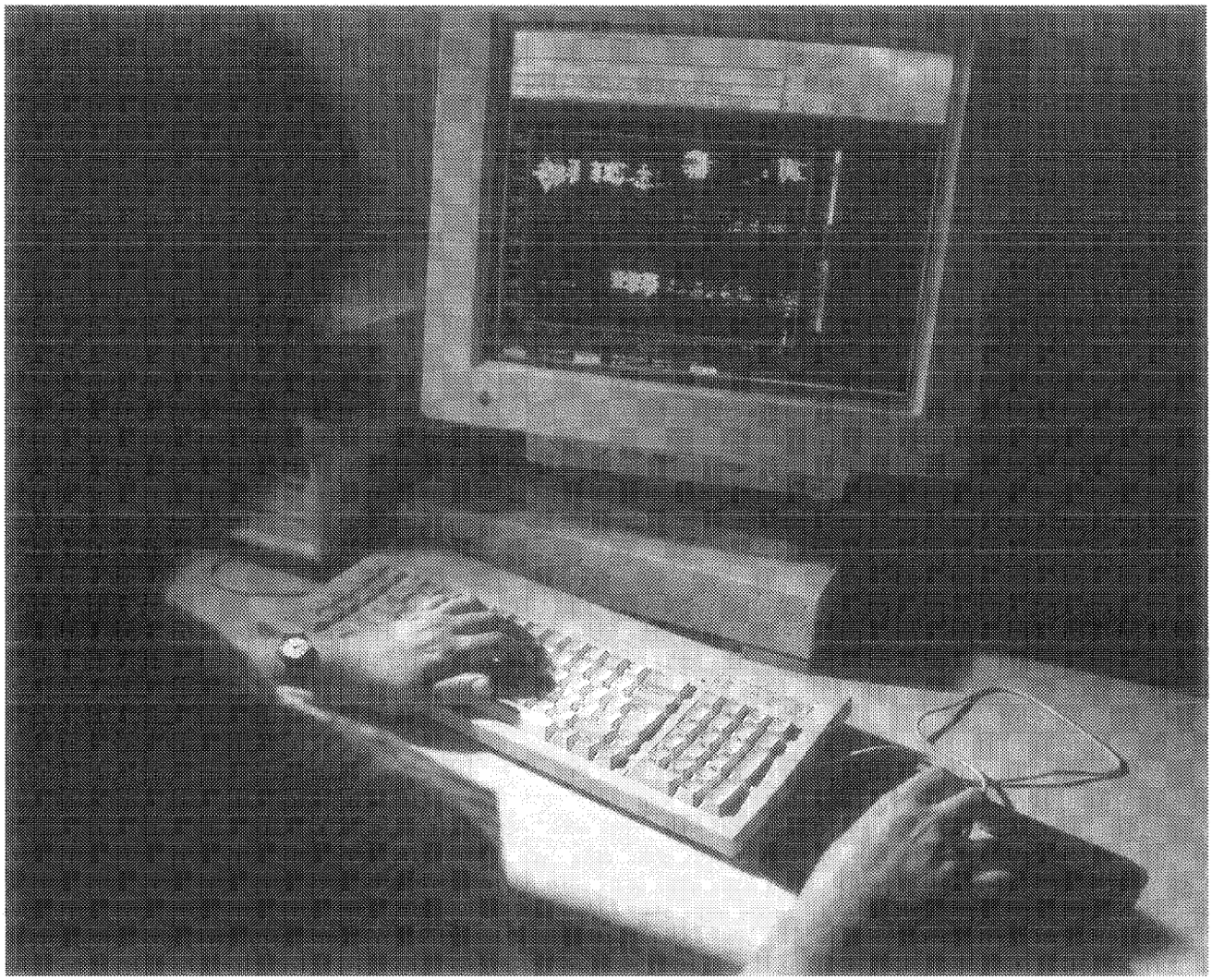
The 500MHz NMR spectrometer recently installed at NIBSC. The computer attached to the spectrometer allows the spectrum of the NMR sample to be analysed. This equipment can be used to confirm the identity and assess the purity of many biological medicines, and thus ensure the highest standards in future control testing.



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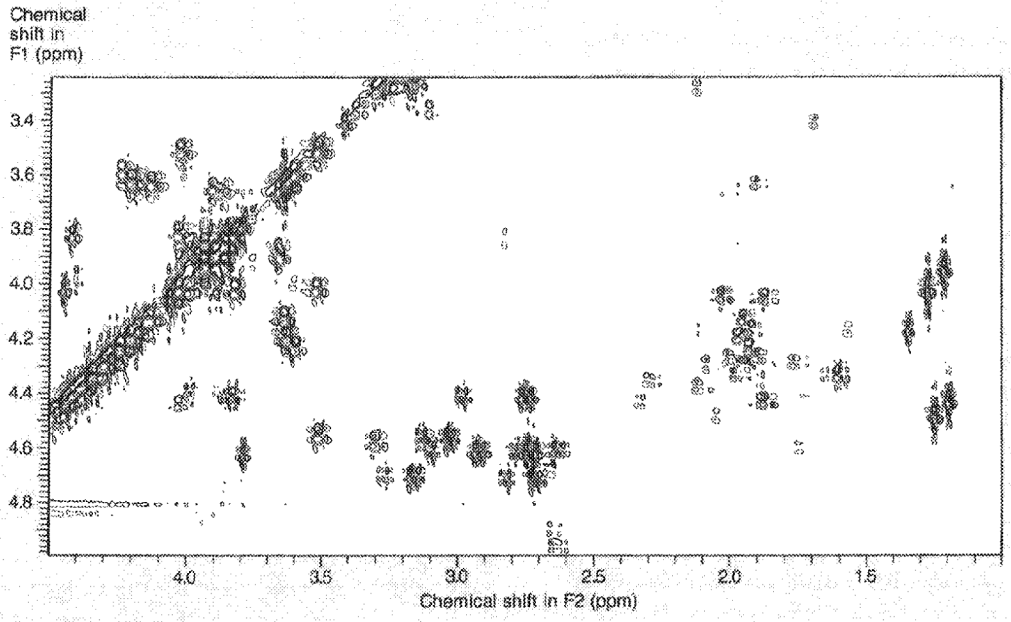

FIGURE 5
Partial 500MHz NOESY spectrum of an anti-coagulant (recombinant desulphatohirudin). Such spectra give fingerprints for biological medicines that can be used to detect minute but clinically significant variations between preparations.

In January 1992 a 500MHz nuclear magnetic resonance (NMR) spectrometer was installed in the newly-formed Laboratory of Molecular Structure (see illustration). The spectrometer facilitates detailed structural studies of proteins. Molecular modelling capabilities have been upgraded within the Laboratory and collaboration with the Informatics Laboratory has led to the development of computer programs to aid the detailed analysis of interpreted spectra.

Current Applications

NMR spectroscopy associated with carbohydrate structure has already been used extensively to confirm structure (and hence serotype) and assess purity of bacterial vaccines (eg those for meningitis). Structural studies of the carbohydrate chains of glycoprotein hormones are also underway.

A series of projects to study the protein structure of recombinant hormones, cytokines and other biologicals has been planned. These projects will improve future control testing by relating structure and conformation to biological activity - if close correlation exists, then structural studies could help to assess potency and purity as well as to identify the biologicals themselves (see Figure 5).

Future Developments

Circular dichroism techniques will be used to compare the conformation of recombinant proteins (products of biotechnology) with native protein material.

Another complementary technique is electrospray mass spectrometry (ESMS), to which NIBSC has limited access. ESMS allows a rapid check on the identity of a protein, reveals structural modifications or degradation that may have occurred during processing and shows the presence of impurities.

The range of techniques mentioned above can in future be used in the control of a wide variety of samples of new products. It is hoped that it may ultimately be possible to replace certain more expensive and less imprecise *in vivo* methods of control.

Physicochemical methods provide structural information not hitherto available, and taken together with traditional biological methods they will allow a much more comprehensive assessment of the quality of a product, to the benefit of public health.

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Perspectives for future development

Scientific developments

The rate of scientific progress in medicine and biology and in the industrial application of modern technologies over the past decade has been striking and continues to gain momentum. New biological medicines derived from recombinant DNA technology and monoclonal antibody methods are coming forward for licensing at an increasing rate. New strategies for the design and use of medicinal products, such as gene therapy, also create new scientific challenges.

Developments in the pharmaceutical industry for manufacturers and NIBSC alike need to be paralleled by scientifically based and judiciously applied regulatory developments if public health is to benefit fully from the new biological medicines. NIBSC's leading scientific role helps to minimise delays in the process of bringing medically valuable products into full clinical use, as well as ensuring the safety and efficacy of such products.

Modern developments in biotechnology are leading to major improvements in the technology applied to the quality control and standardisation of both conventional and novel biological medicines, and thus enhance the public health value of these products.

There will be an increasing need for such technical innovations in order to control products of increasing sophistication.

NIBSC developments

NIBSC has responded to these new challenges by redeploying staff and setting up new scientific units focusing on Immunobiology, AIDS, Molecular Structure and Informatics. These developments represent a considerable investment by the UK Government and have placed NIBSC in an excellent position to meet the challenges of the new era of biotechnology and those arising from the pharmaceutical legislation of the EC.

During the next ten years developments in the pharmaceutical industry are likely to yield new products and approaches in a number of specific areas which will require a concerted response from NIBSC to ensure the highest standards of product and the availability of biological standards.

Cytokines, growth factors and hormones

These substances currently represent a rapidly growing area of the field of biological medicines. Each new product presents new sets of issues for standardisation and control. Applications for licensing of novel cytokines and hormones are likely to occur regularly in the future.

New vaccines

Recombinant-DNA technology provides opportunities for the precise genetic modification of bacteria and viruses to produce living vaccines against diseases where existing vaccines are expensive, ineffective or non-existent. The standardisation, control and public health aspects of the safety of these vaccines are particularly complex, and will account for a substantial proportion of NIBSC's work during the next decade.

The effects on public health of the new generation of vaccines will be considerable and they will play a major role in immunisation strategies both nationally and internationally (through the WHO's Expanded Programme of Immunization and Vaccine Development Programme and the International Children's Vaccine Initiative (CVI)).

The development of vaccines against AIDS is a highly complex field. Successful vaccine development will probably depend upon the application of new concepts of vaccine design and recombinant technology. Slow but sustained progress is currently being made in this field but it may be many years before such products are available. The standardisation and control of these vaccines will be highly demanding. NIBSC is actively involved in research on vaccines against HIV in collaboration with the Medical Research Council, the EC and the World Health Organization, an involvement that is seen as essential for building the capacity to carry out effective control testing in the future.

Important scientific developments in the field of parasitic disease are leading to the development of effective vaccines. For example,

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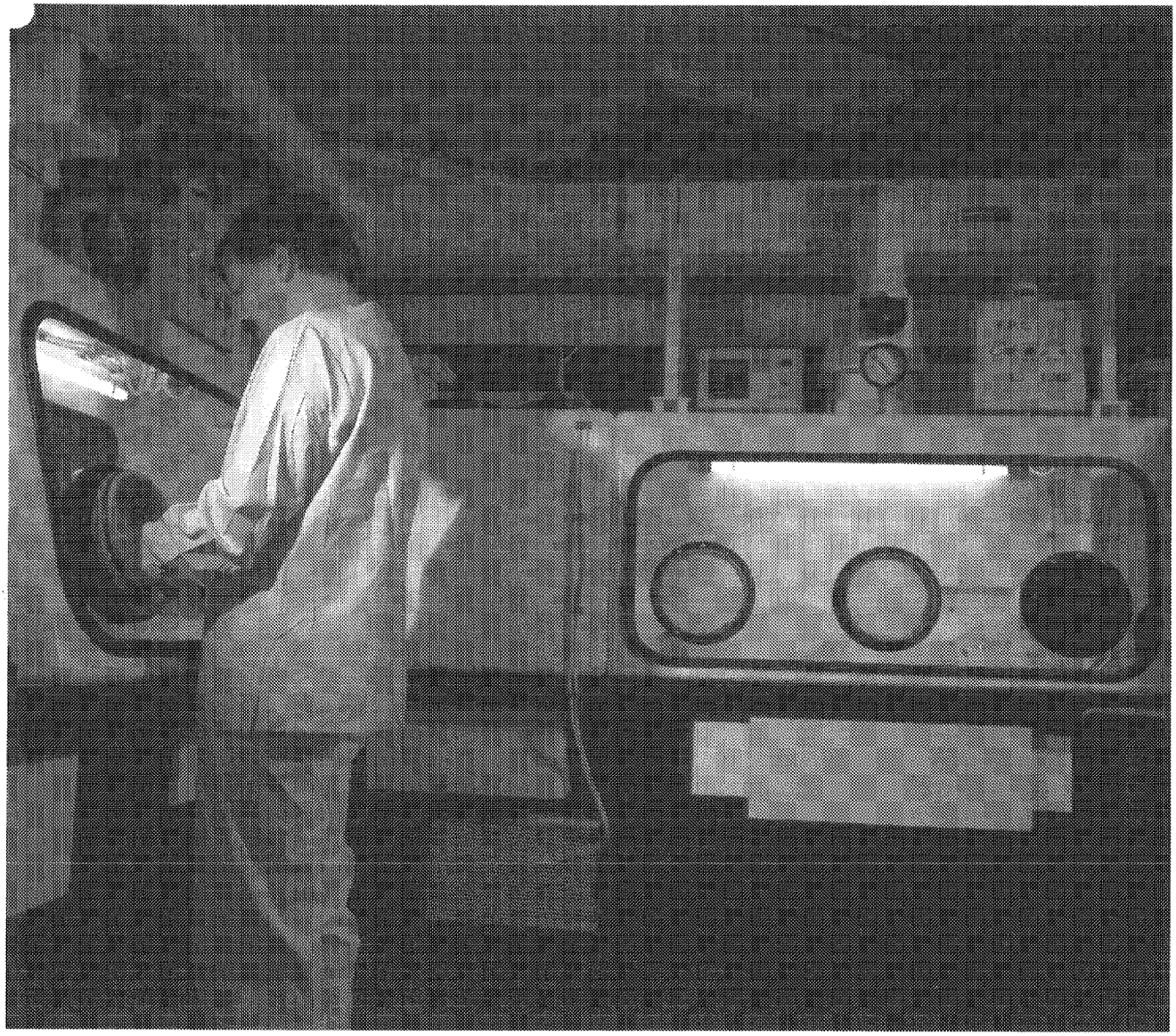

NIBSC is equipped with special containment facilities which enable scientists to work safely with highly infectious agents.

experimental vaccines against malaria are now being evaluated in some countries. New techniques of control and standardisation will be required.

Diagnostics

Progress in the development and use of *in vitro* diagnostics has been rapid in recent years. Many are based on advanced biotechnology and on the use of biologicals.

To date it has been possible to devote only limited resources to standardisation in this field. But as diagnostics and their use proliferate, NIBSC may be required to effect the control and standardisation of the biologicals used as future diagnostics, if not of the diagnostic kits themselves, particularly for those classes of diagnostics that may be subject to EC Directives.

Gene therapy

Techniques are now available for the delivery of specific genes into the somatic cells of patients with various diseases. These may include human genes designed to remedy genetic deficiencies such as cystic fibrosis or genes designed to combat pathologies such as cancer. This new area of medical science is currently in the laboratory phase, but is likely to reach practical application in the form of therapeutic biological products in the next few years and some clinical trials are already in progress. Gene therapy technology will depend on the use of complex gene delivery systems such as retroviruses. The regulatory issues will be complex and NIBSC will be in a position to play a major role in providing advice and testing the products.

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Summary accounts

Income and expenditure account

for the year ended 31 March (Subject to audit)

	1991/92 £000	*1990/91 £000*
INCOME		
Government Grants	8,456	7,933
Other Grants	99	97
Income from Activities	759	441
Transfer from Reserves	1,507	1,272
	10,821	9,743
EXPENDITURE		
Staff Costs	5,647	5,285
Depreciation	1,507	1,272
Other Operating Charges	3,643	2,989
	10,797	9,546
Operating Surplus	24	197
Extraordinary Expenditure re. Settlement of Contractor's Claim	115	
	(91)	
Interest receivable	29	41
Surplus/(Deficit) on ordinary activities	(62)	238
Surplus/(Deficit) brought forward	60	(178)
Surplus/(Deficit) carried forward	(2)	60

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Balance sheet

(Subject to audit)

	31.3.92 *£000*	*31.3.91* *£000*
FIXED ASSETS		
Tangible Assets	20,955	19,497
CURRENT ASSETS		
Stock	139	136
Debtors	1,078	595
Cash at bank and in hand	551	715
	1,768	1,446
CREDITORS		
Amounts falling due within one year	1,067	674
Net Current Assets	701	772
Total Assets Less Current Liabilities	21,656	20,269
Financed by:		
Deferred Grant Income	155	107
Deferred Government Grant	5,337	3,131
Capital and Reserves		
Capital Reserve	16,166	16,971
Income and Expenditure Account	(2)	60
	21,656	20,269

Notes:

1. **Staff numbers**
   The average number of employees in 1991/92 was 301 (288 in 1990/91).

2. **Capital expenditure:**
   The total capital expenditure in 1991/92 was £2,966,000 (£842,000 in 1990/91). £1,480,000 of the expenditure was the final payment for building the NIBSC laboratories.

3. Deferred Government Grant and Capital Reserve contain provisions to cover depreciation of assets in future years.

4. A copy of the full annual accounts can be obtained from the Director of NIBSC.

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Board membership

The National Biological Standards Board (NBSB) is accountable to the Secretary of State for Health for the management of NIBSC. The membership of NBSB during 1991/92 was as follows:

Dr N J B Evans, CB MA FFCM DPh (Chairman)
Professor J S Beck, BSc MB ChB MD
Professor S R Bloom, MA DSc MD FRCP
Dr C J Coulson-Thomas, MSc(Econ) MA MSc AM MPA DPA PhD FCA FCIS
Mr D F R Crofton, BComm FCA ACIS
Dr M Ferguson, BSc PhD
Professor K Gull, BSc PhD
Professor H S Jacobs, MD FRCP
Mr P J S Lumsden, MA FCA FCT
Dr R M Maskell, MA DM FRCP
Mrs N Morris, MA
Professor D K Peters, MB BCh FRCP
Mr J Pring, Dip.RCP Dip.RCS
Dr G C Schild, BSc PhD FIBiol
Professor J G Ratcliffe, MSc DM BMBCh FRCP FRCPath
Dr J R Tata, DSc FRS
Professor R A Weiss, BSc PhD

For further information on the work of NIBSC, please contact:

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