tal temperature, irritability, respiratory symptoms and local reactions (redness, soreness, etc.).

Serology. Blood samples were taken at the ages of 3, 4, 6, 12 and 14 months. Anti-PRP antibody levels were assayed at the National Public Health Institute, Finland, using a Farr-type radioimmunoassay.[21,22] The minimal amount of antibody detectable was 0.06 μg/ml.

Anti-diphtheria antibody assays were performed by a microcell culture method based on the neutralization of toxin killing of Vero cells. The reference antiserum was an NIH standard antitoxin containing 6 IU/ml.[23] Anti-tetanus antibodies were assayed by a solid phase enzyme-linked immunosorbent assay using tetanus toxoid as the capture antigen and protein A as the detecting reagent.[24] The reference antiserum contained 5.35 IU/ml as determined by a toxin neutralization test in mice. Both diphtheria and tetanus toxoid antibody assays were performed at the Connaught Research Institute, Toronto, Canada.

Poliovirus antibodies were measured at the National Public Health Institute, Finland, using the microneutralization method in Vero cells.[25,26] The test strains were poliovirus type 1/Brunhilde, type 2/MEF and type 3/Saukett.

Statistical methods. One-way analysis of variance and linear correlation analysis of log-transformed data were used to compare geometric mean antibody titers and to test for possible associations between the antibody titers in different groups.

RESULTS

Adverse reactions. The most commonly reported adverse effect was irritability which was reported after 49% of the sessions during which PRP-D was given (Table 1). However, the same was true in 53% of DTP and/or IPV vaccinations at same ages. The incidence of fever and local reactions was slightly higher in the group receiving PRP-D in addition to the routinely scheduled vaccines. The highest fever measured was 39.3°C rectally, seen in one child after PRP-D plus DTP plus IPV vaccination. There was no correlation between the rate of adverse reactions and the level of PRP or diphtheria toxoid antibodies before injection.

Serology. The PRP antibody levels were low up to the age of 6 months even in children who had received two doses of PRP-D. However, after a third injection antibody levels increased to 0.35 μg/ml at 7 months. A fourth injection given at 12 months acted as a further booster, raising the antibody level to 4.87 μg/ml (geometric mean titer).

Simultaneously administered PRP-D had no effect on the antibody responses to diphtheria toxoid, tetanus toxoid or the polioviruses (Table 2); all P values were ≥0.5. In a linear correlation analysis, final PRP antibody levels at 7 months did not correlate with

TABLE 1. Adverse reactions reported at any time during first 48 hours after vaccination

	Age 3 Months		Age 4 Months		Age 6 Months		Age 12 Months	
	DTP (N = 25)	DTP + PRP-D (N = 25)	DTP (N = 23)	DTP + PRP-D (N = 25)	DTP + IPV (N = 23)	DTP + IPV + PRP-D (N = 24)	IPV (N = 22)	IPV + PRP-D (N = 22)
Local redness	0	2	0	1	1	1	1	0
Local soreness	1	3	1	4	1	1	0	0
Fever (≥38.5°C)	4	2	0	5	6	6	0	1
Irritability	17	17	12	12	12	11	8	7
Sleepiness	0	0	0	0	0	0	0	0

TABLE 2. Geometric mean antibody levels of infants receiving DTP at 3, 4 and 6 months and IPV at 6 and 12 months with or without PRP-D at 3, 4 and 6 months

	3 Months	4 Months	6 Months	7 Months	12 Months	14 Months
PRP (μg/ml)						
With PRP-D	0.08	0.08	0.10	0.35	0.19	4.87
Without	0.10	0.08	0.07	0.07	0.10	0.11
Diphtheria antitoxin (IU/ml)						
With PRP-D	0.010	0.005	0.036	0.411	ND[b]	ND
Without	0.014	0.005	0.031	0.352	ND	ND
Tetanus antitoxin (IU/ml)						
With PRP-D	0.049	0.109	0.612	3.666	ND	ND
Without	0.052	0.113	0.430	3.688	ND	ND
Polio 1[a]						
With PRP-D	7.1	<4	<4	8.7	5.7	370
Without	12	8.2	4.8	7.1	6.1	320
Polio 2[a]						
With PRP-D	28	17	7.5	24	16	230
Without	18	10	5.5	12	6.3	270
Polio 3[a]						
With PRP-D	4.5	<4	<4	18	6.5	210
Without	4.7	<4	<4	18	4.3	290

[a] Reciprocal of end point dilution neutralizing 50 to 100 $TCID_{50}$ (50% tissue culture infectious dose) units of virus.
[b] ND, not determined.

diphtheria antibody levels (Pearson's correlation coefficient, 0.15; $P = 0.49$).

DISCUSSION

The study design permitted us to investigate the immune responses to the vaccines routinely used in Finland, given either alone or together with PRP-D. In these two groups of children, we measured serum antibodies to diphtheria and tetanus toxoids and to inactivated polio vaccine by routine methods. The answer was clear-cut. PRP-D did not interfere with the antibody response to routine vaccines at any time point. Also the reactogenicity of these vaccine combinations appears to be comparable to that of DTP or IPV given alone.

Pertussis antibodies were not determined because of lack of uniformly accepted standards of relevant pertussis antigen and antibody determinations. However, we have no reason to believe that the response to pertussis antigens should be different. The study gives no answer to the question of the suitability of PRP-D and live viral vaccines because they are not used during infancy in the Finnish vaccination schedule.

PRP antibody concentrations in the PRP-D-vaccinated children were somewhat lower than those reported earlier.[15] This may be a result of both the different vaccine lot and the different vaccination schedule.[16] In an earlier study with unconjugated vaccine the anti-PRP antibody concentrations were lower when DTP vaccine was administered simultaneously with PRP compared with concentrations after PRP alone.[27] On the other hand in two other studies the opposite was true,[12,13] and in a recent study in Finland we could not detect any significant inhibitory effect.[14] Those data are, however, not directly comparable to the present study in which a PRP conjugate was used. In animal studies both the anti-PRP and anti-toxoid antibody concentrations were increased when Hib capsular polysaccharide conjugates were given concurrently with the relevant tetanus or diphtheria or DTP.[19,26] We have no data on the possible effect of DTP on PRP responses because all children receiving PRP-D also received DTP.

In summary the results obtained show that Hib vaccine can be incorporated in the normal vaccination programs, to be given together with DTP or IPV, without the need to increase the number of visits to the child health centers.

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Outbreak of enterovirus 71 infection in Victoria, Australia, with a high incidence of neurologic involvement

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An outbreak of infections caused by enterovirus 71 occurred in southeastern Australia during the winter of 1986. Infection was confirmed by virus isolation or serology in 114 patients, 65 of whom were admitted to hospital. Fifty-one percent of inpatients were infants younger than 12 months old and 85% were younger than 5 years old.

Many cases of hand, foot and mouth disease occurred in the community during the epidemic, but 51% (33 of 65) of patients admitted to hospital had central nervous system involvement, often associated with severe symptoms. Six patients had encephalitis and one had a poliomyelitis-like paralytic illness. Various skin manifestations other than hand, foot and mouth disease occurred, especially in young children, and 25 patients had significant respiratory disease including at least 7 with pneumonia.

Enterovirus 71 is one of very few viruses that cause hand, foot and mouth disease as well as a variety of other clinical manifestations. The most important of these is meningoencephalitis, which causes significant morbidity, especially in infants and young children.

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INTRODUCTION

The first recognized cases of disease caused by a new enterovirus, which was subsequently called enterovirus 71 (E71), occurred in California between 1969 and 1972.[1] Most of the patients, from whom the virus was isolated, had benign central nervous system (CNS) infection. However, one isolate was from the brain of a child who had died of encephalitis. In the southern summer of 1972 to 1973 the same virus caused an epidemic in Melbourne.[2] Aseptic meningitis was the commonest manifestation but some patients had vesicular rashes or respiratory symptoms. A similar outbreak occurred in Sweden during the subsequent northern summer.[3] Widespread epidemics of hand, foot and mouth disease and aseptic meningitis

NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT
BILTHOVEN, THE NETHERLANDS

Report no. 213675005

Pienter project: description of serumbank and information on participants from the questionnaires

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This investigation has been performed by order and for the account of the Ministry of Health, Welfare and Sports within the framework of project no. 213675, Serosurveillance
Dit onderzoek werd verricht in opdracht van de Inspectie voor Gezondheidszorg en ten laste van het ministerie van Volksgezondheid, Welzijn en Sport in het kader van project nr. 213675
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218	Bureau Rapportenregistratie
219-250	Bureau Rapportenbeheer
251-300	Reserve

ABBREVIATIONS

CBS	Centraal Bureau voor de Statistiek / Central Bureau of Statistics
NIP	National Immunisation Programme
RIVM	Rijksinstituut voor Volksgezondheid en Milieu / National Institute of Public Health and the Environment
SAS	Statistical Analysis
SOP	Standard Operating Procedure
TNO	Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek / Dutch Organization for applied scientific research
SES	Social Economic Status
CIE	Centrum voor Infectieziekten Epidemiologie / Centre for Infectious Diseases Epidemiology
PHS	Public Health Service
COPD	Chronic Obstructive Pulmonary Disease

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SAMENVATTING

Doel In oktober 1995-december 1996 is het Pienter project uitgevoerd. Doel van het project was de opzet van een representatieve serumbank van de algemene Nederlandse bevolking voor sero-epidemiologische studies, met name ter evaluatie van het Rijksvaccinatieprogramma (RVP).

Opzet In het Pienter project werden sera en vragenlijsten verzameld van de algemene Nederlandse bevolking (0-79 jaar) door middel van een cross-sectioneel populatie-onderzoek, inclusief een non-respons onderzoek.

Methoden Binnen vijf geografische regio's met vergelijkbare inwoneraantallen is een naar inwonertal gewogen steekproef getrokken van acht gemeenten. In aanvulling daarop werden acht gemeenten met een lage vaccinatiegraad gekozen welke van belang zijn in ons land door bepaalde religieuze groeperingen die vaccinatie weigeren. Uit de 48 gemeenten werd een naar leeftijd gestratificeerde steekproef getrokken van 380 personen. De steekproef bestond uit 17 leeftijdsklassen (0, 1-4, 5-9 tot en met 75-79 jaar). Uit de eerste twee leeftijdsklassen werden 40 personen geselecteerd, uit de volgende 20 personen. De uitgenodigde personen werden gevraagd een spreekuur te bezoeken voor bloedafname en een vragenlijst in te vullen. De vragenlijst bevatte gegevens over demografische variabelen (sexe, nationaliteit etc), vaccinatie (deelname aan het RVP, (noodzaak van) vaccinaties tegen DKTP, BMR, Hib, vaccinatie tegen DTP, tetanus, hepatitis A, hepatitis B, griep), religie, reizen, langdurig hoesten, contact met zoet oppervlaktewater, contact met dieren, seksueel overdraagbare aandoeningen, bloeddonorschap, beleving van eigen gezondheid, chronische ziekten, rook- en drinkgewoonten, beroep en opleiding. Personen die niet bereid waren bloed te geven werden verzocht alleen de (verkorte non-respons) vragenlijst in te vullen. Dit rapport beschrijft de informatie over de deelnemers aan het Pienter project op basis van gegevens uit de vragenlijst. Het betreft deelnemers van wie zowel bloed als een ingevulde vragenlijst werd ontvangen.

Resultaten en conclusies. Een serumbank met 9948 monsters is beschikbaar voor vele sero-epidemiologische studies. Alle GGD's en gemeenten hebben enthousiast meegewerkt aan het Pienter project. De respons was 55% (n=8359) in de nationale steekproef en 53% (n=1589) in gemeenten met een lage vaccinatiegraad. Vrouwelijke deelnemers, deelnemers met een lage SES, deelnemers met de Nederlandse nationaliteit en deelnemers geboren in Nederland waren iets oververtegenwoordigd in het Pienter project, vergeleken met cijfers van het CBS. De deelnemers waren representatief voor de Nederlandse bevolking qua burgerlijke staat, religie en gezondheid, vergeleken met cijfers van het CBS. De informatie van non-participanten maakt het mogelijk om seroprevalenties te corrigeren voor mogelijk selectieve non-respons. De deelnemers gaven aan vaccinatie tegen polio het meest belangrijk te vinden. Vaccinatie tegen difterie, tetanus, kinkhoest en Hib werd ongeveer even belangrijk gevonden en vaccinatie tegen mazelen, bof en rode hond werden minder belangrijk gevonden. Bevindelijk gereformeerde deelnemers gaven aan de verschillende vaccinaties uit het RVP minder belangrijk te vinden dan deelnemers van de gereformeerde bond en deelnemers met geen religie of een religie zonder bezwaren tegen vaccinatie en zij gaven aan minder vaak deel te hebben genomen aan het RVP. Respondenten van de gereformeerde bond en de bevindelijk gereformeerde deelnemers uit de landelijke steekproef gaven vaker aan te hebben deelgenomen aan het RVP en gaven vaker aan de vaccinaties uit het RVP belangrijk te vinden dan die deelnemers uit de lage vaccinatiegraad gemeenten. Interessant om te onderzoeken is of de verschillen in (mening over) vaccinatie die gevonden werden tussen de verschillende religieuze groeperingen en tussen de landelijke steekproef en de lage vaccinatiegraad gemeenten ook gereflecteerd worden in de seroprevalenties voor ziekten waartegen vaccinatie beschikbaar is. Over deze seroprevalenties zal separaat worden gerapporteerd.

SUMMARY

Introduction The RIVM has carried out the so-called Pienter project in October 1995 - December 1996. The aim of this study was to establish a serum bank of a representative sample of the Dutch population to facilitate sero-epidemiological studies mainly for the evaluation of the National Immunisation Programme (NIP).

Design Collection of sera and questionnaires from the Dutch population (0-79 years) through a cross-sectional study, including a non-response survey.

Methods The Netherlands were divided in five regions with comparable numbers of inhabitants. Within each region a sample was drawn of eight municipalities, weighted by the number of inhabitants. An additional sample of eight municipalities with a low immunisation coverage was chosen which are of particular interest in our country with specific religious groups who refuse vaccination. Within each municipality an age-stratified sample of 380 persons was drawn from the population register. The sample consisted of 17 age strata (0, 1-4, 5-9 to 75-79 years). In the first two strata 40 individuals were sampled while in each of the following strata 20 individuals were sampled. The participants were invited to a consultation hour for blood sampling and to fill in a questionnaire. The questionnaire contained data on demographic variables (gender, nationality etc.), vaccination (participation in the NIP, (necessity on) vaccination against DTP-IPV, MMR, *Hib*, vaccination against DTP, tetanus, hepatitis A, hepatitis B, influenza), religion, travelling, longterm coughing, recreation in fresh waters, contact with animals, sexual transmitted diseases, blood donation, self perception on health, chronic diseases, smoking and drinking habits, occupation and education. Persons who refused were asked to just fill in the questionnaire or in second instance to answer some questions for the non-response survey. This report describes the information on the participants of the Pienter project based on data from the questionnaire. It concerns participants of whom both blood and a completed questionnaire was received.

Results and conclusions. A serumbank of 9948 samples has been established which can facilitate many sero-epidemiological studies. All Public Health Services and municipalities have co-operated enthusiastic. The overall response was 55% (n=8359) in the national sample and 53% in the low immunisation coverage sample (n=1589). Participants of the female sex, with a low SES and participants with the Dutch nationality and participants born in the Netherlands were somewhat overrepresented in the Pienter project compared with figures from the Central Bureau of Statistics (CBS). The participants were representative for the general Dutch population for marital status, religion and health compared with figures from the CBS. The information on the non-participants offers the opportunity to correct the seroprevalences for possible selective non-participation. The participants thought immunisation against poliomyelitis was most important, then diphtheria, tetanus, pertussis and hib and immunisation against rubella, mumps and measles was considered less important. Participants belonging to the orthodox reformed thought the different immunisations from the National Immunisation Programme (NIP) were less important and participated less in the NIP in comparison with participants of the reformed bond and participants with no religion or a religion not opposed to vaccination. Participants of the reformed bond and of the orthodox reformed in the national sample participated more in the NIP and thought immunisations from the NIP were more important though than those participants in the low immunisation coverage sample. Interesting to study is whether the differences in (opinion on) immunisation found between the various religious groups and between the low immunisation coverage sample and national sample are also reflected in the seroprevalences of diseases for which vaccination is available. Separate reports will be published on these seroprevalences.

ACKNOWLEDGEMENT

The establishment of a representative serum bank of the Dutch population with about 10.000 sera could only be realised through the elaborate co-operation of many parties: the Public Health Services who mediated between municipalities and participants on one hand and the RIVM on the other and facilitated the data-collection, the municipalities that drew the sample from the population register, the administrative Pienter team who managed to get the mailing out in time every week and handled thousands of phone calls, the field staff of the Pienter project who tried to comfort all participants and collected all blood samples, the Laboratory for Infectious Diseases Diagnosis and Screening (LIS) who offered the facilities to process the blood into serum, the Pienter laboratory team that processed all the blood samples and last but not least the participants without whom this project never could have been realised.

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1. INTRODUCTION

The National Institute of Public Health and the Environment (RIVM) collects morbidity and mortality data on several diseases in order to assess the national health status. These data are used for signalling problems and trends and evaluating the pursued public health policy. For the surveillance of infectious diseases in addition to notification data also important information can be derived from laboratory results, especially for diseases where the laboratory is often used for setting a diagnosis. But also important information can be derived from the assessment of specific antibodies in serum of healthy individuals since they mark undergone clinical ánd subclinical diseases. For the diseases from the National Immunisation Programme (NIP) this is called immunosurveillance; measurement of specific antibodies will give insight into the protection (immune status) of the population against diseases which are included in the NIP.

The NIP was implemented in 1952 in the Netherlands with catch-up campaigns for the cohort born in 1945-1951. The NIP provides vaccination for all children in the Netherlands against the following diseases: diphtheria, tetanus, pertussis and poliomyelitis (DTP-IPV), mumps, measles and rubella (MMR) and *Haemophilus influenzaee* type b (Hib). Through the intervention of the NIP, incidences of diseases against which is vaccinated have decreased dramatically. However, despite the high vaccination coverage, epidemics of measles and pertussis still occur (1,2). The occurrence of diphtheria in the future is also possible (3). Therefore continuous control of the effects of the NIP remains important (4).
The epidemiological dynamics of infectious diseases can change under the influence of vaccination. The force of infection will decrease and this will result in delay of unprotected (unvaccinated) individuals getting infected. This increase of the mean age of infection can result in a higher chance of clinical disease and complications. This could implicate that vaccination increases the risk of complications for unvaccinated individuals.
To assess the long-term effects of mass vaccination insight into the (possible change of) duration of both vaccine-induced immunity and natural immunity is necessary. It could be that one can no more rely on lifelong persistence natural immunity in contrast to the past. When no or less circulation of the pathogen occurs, the lower force of infection results in lack of boosting of both natural and vaccine-induced immunity. Also the protection of newborns by maternal antibodies may be of shorter duration.
In addition, despite the high vaccine-coverage, the vaccination will never reach the whole population since some groups, such as several religious groups, refuse vaccination. If non-immunised individuals are social-demographically clustered the herd immunity can be broken. As a result epidemics can occur. This for example took place in the Netherlands during the polio epidemic in 1992/1993 (5). This epidemic was restricted to religious groups who refuse vaccination and are living in the so-called 'bible belt', a geographic band over the country

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where communities with low vaccination coverage are found. Also the high incidence of congenital rubella syndrome after a rubella epidemic among the Amish people in the United States demonstrates this effect. Due to the low vaccine coverage and social clustering in combination with the absence of regular contacts outside their own community the number of susceptible individuals could increase (6).

The RIVM has undertaken the so called Pienter project (Peiling Immunisatie Effect Nederland Ter Evaluatie van het Rijksvaccinatieprogramma). The aim of this study was to establish a serum bank of a representative sample of the Dutch population for the purpose of public health research (7).
The main purpose of the Pienter project was to evaluate the NIP. Therefore the immunity of the population for the diseases against which its is immunised, has to be guarded. This can be done through immuno- or serosurveillance: the study of the prevalence of specific antibodies. On the basis of serological profiles, clusters with high amounts of susceptible individuals in certain age, social or geographical groups can be recognized. Adjustments in the NIP can be considered to prevent elevations of morbidity and mortality.
Other aims of the study were to gain insight into the occurrence of infectious diseases with a frequent subclinical course and into the prevalence of serum determinants of other (chronic) illnesses. Serologic data hold important information on other infectious diseases too.
Surveillance of clinical cases alone is not enough since a lot of infectious diseases are known to have an asymptomatic course. On the basis of age-specific seroprevalence, rates of transmission can be estimated.
Although the costs of the establishment of a representative serumbank are high, other well-based studies can be undertaken with the collected sera since antibody titers remain stable when stored under the right circumstances (8). This way, the serumbank can be used for a lot of surveys on seroprevalence of other diseases than those from the NIP. Therefore, the questionnaires are set up very broad which means that it contains questions on a lot of general risk factors, so that later associations with specific seroprevalences can be studied. Still, this does not mean that every relevant question regarding a specific disease is included. Choices had to be made.

The study was designed as an alternative for prior carried out immunosurveillance studies against which poor representivity was the main objection. Therefore, this population-based study used random sampling rather than selections of individuals, e.g. individuals who visit a general practitioner. Because of the high costs and many involved organisations, a pilot-study was executed in four cities in the province Utrecht. This pilot was performed in 1994 to test the feasibility and obtain insight in reasons for non-participation so measures could be taken to achieve a higher response in the main study and to test representivity. The results of that pilot were so encouraging that it was decided to set up a national study conform the model of the

pilot, except for some minor changes in the questionnaire and approach, which were described earlier (9, 10, 11, 12, 13).

This report gives a description of the serumbank and information on the participants of the Pienter project who filled in a questionnaire ánd gave blood. Some comments are given, but this report is mainly meant as documentation, which will be further used when the true outcome variables of the project, i.e. the seroprevalence data, are available for analysis.

2. METHODS

In the Pienter project sera and questionnaires were collected from the general Dutch population (0-79 years) through a cross-sectional study, including a non-response survey.

2.1 Sampling

In the Pienter project, samples were drawn by means of a two-stage cluster sampling technique. The reliability of estimates of seroprevalence is mainly determined by the amount of clusters (municipalities) in the sample and less by the amount of participants per cluster (14). Therefore the highest amount of municipalities possible considering logistics and costs was chosen. On the basis of that finding, it was decided to sample 40 municipalities and 380 persons per municipality, leading to 15,200 invited persons in the national sample.

For that purpose, the Netherlands were divided in five regions with approximately equal numbers of inhabitants (*Table 1*).

Table 1 Zoning of the Netherlands into five regions for stratified sampling of municipalities

region	provinces	number of inhabitants (x1.000, 1 January 1996) (15)
North-East	Groningen, Friesland, Drente and Overijssel	2,681.4
North-West	Noord-Holland and Flevoland	2,741.2
South-West	Zuid-Holland and Zeeland	3,700.3
Central	Utrecht and Gelderland	2,946.9
South-East	Noord-Brabant and Limburg	3,424.1

Per region, municipalities were randomly sampled, weighted by the number of inhabitants. The first eight municipalities with consequent Public Health Services were asked to participate. When a municipality or Public Health Service would refuse or drop out, the next municipality or Public Health Service on the list would have been approached. Within each municipality, an age-stratified sample of 380 persons was drawn from the population register. The sample consisted of 17 age strata, namely 0, 1-4, 5-9, 10-14 till 75-79 years. In the first two strata 40 individuals were sampled while in the each of the following strata 20 individuals were sampled; this oversampling was based on the expected lower response (25% instead of 50%) of very young children.

In addition, this study was carried out in eight municipalities with a low immunisation coverage. The purpose of this additional data collection was to have access to more non-vaccinated individuals, who are of particular interest for the evaluation of the NIP. In the national sample

alone this number would be too small to be able to estimate the seroprevalence of this subgroup.
Municipalities were listed in order of vaccination coverage for the DTP-IPV)-vaccination of the NIP on 1 January 1993. On the basis of a consistently lower vaccination coverage from 1982 to 1993 and the condition of representation of several provinces, eight municipalities were chosen in this additional 'sample'. Sometimes, for logistic reasons (place of consultation hours, accessibility in low density areas), the sample was restricted to residential precincts with an even lower coverage. Although these municipalities were chósen, the sampling of persons from the population registers was randomly anyway. Further, the procedure was the same as for the national sample so that 3.040 persons were invited to participate in the low immunisation coverage sample. In Appendix I the 48 municipalities are given.

2.2 Co-operation with Public Health Services

The Public Health Services (PHS's) were chosen as partners in this project because of their public health tasks, which are comparable with those of the RIVM. Furthermore they have expertise on research in the general population and they are a well-known organisation to the people.
In June 1995 an introductory meeting was organised at the RIVM for the Public Health Services in whose districts one or more municipalities of the random sample were present, in order to inform them about the Pienter project. The three reports written on the pilot-study (9) were sent to them before the meeting.
As the data collection was carried out municipality by municipality and covered a period of 15 months in total (October '95 - December '96), every PHS was contacted again about four months before the data-collection in that particular municipality would start. In this meeting the following subjects were covered:

- backgrounds of the Pienter project
- sampling procedure
- activities expected of the PHS
- contact person at the PHS
- special groups in the municipality (e.g.allochtonous persons)
- anonymity of the participants
- co-operation contract
- availability of local study results for the PHS
- time schedule
- willingness of PHS to co-operate

Besides the infectious diseases physician of the PHS, the infectious diseases nurse, the epidemiologist, the head of the general health care department(Algemene Gezondheidszorg) and/or the director were invited to attend the meeting according to the advice of the infectious diseases physician. Before the meeting the PHS was sent a package of study materials including the example draft of the co-operation contract, the Pienter information brochure for participants (annexe 1), the protocol, the invitation letters for the participants (annexe 2), the model of the appointment form (annexe 3), the questionnaires (annexe 4), the models of the informed consent forms for the participants (annexe 5), the model of the letter for the town council, a description of the sample to be drawn from the population register and a standard pressrelease. When the PHS had decided to participate in the Pienter project, the contract (annexe 6) was signed by both parties and the PHS approached the town council of the concerning municipality with the request to participate in the Pienter project and give their approval for drawing a sample from the population register. The PHS informed the RIVM as soon as possible on the decision made by the town council.

2.3 Drawing the sample from the population register

When the town council decided positively on participation, the name of a contact person at the local administration office (mostly an automation expert) was given to the RIVM. The automation expert and the RIVM had directly contact about the sampling without mediation of the PHS. When the municipality could make a PC-dump of their population register, the RIVM offered a computer programme with which the sample could be drawn from the PC-dump. As an alternative the municipality could supply a random sample of the population register (N=4000-5000) from which the RIVM could draw the stratified sample of 380 persons.
The sample had to include the following data on the participants: family name, prefix, maiden name, prefix, initials, date of birth, street, housenumber, postal code (numbers and letters), town, marital status and nationality.
The sample had to be drawn approximately two weeks before the data-collection in the municipality but not much sooner because of possible changes in the sample through deaths and moving persons. The RIVM asked for information on these changes from the contact person of the municipality right before the participants were approached by the staff members of the Pienter project.

Not all municipalities could provide all requested variables from the population register. One municipality could not provide the marital status and one municipality could not provide the marital status and the nationality of its inhabitants.

2.4 Invitations

Three versions of the invitation letters were available: for persons of 0-11, 12-16 and 17-79 years of age. Translation in Turkish and Moroccan were available and were sent to individuals with a Turkish or Moroccan nationality. On the invitation letters the initials, family name, address, town and birth date of the eligible persons were given.
The standard invitation letters was printed on the PHS's stationary at the RIVM and sent to the PHS. The director signed it and sent these back to the RIVM where they were also signed by the director of the division Public Health Research on behalf of the RIVM.
With the help of a SAS programme individual appointments were proposed at times when thought it would suit individuals best (school-going children after school was out, men in their working years at lunch time, old individuals not too early in the morning) and Turkish and Moroccan individuals were invited at days when field workers who spoke Turkish or Moroccan were present. In order of invitation a unique individual number (U-number) was assigned to every invited person.
The date and time of the appointment were printed on appointment forms as were the address of the local health service or other location where the consultation hours would take place, the individual U-number, initials, family name and birth date of the invited person.
The mailing package for the eligible participants contained the invitation letter and the appointment form accompanied by a brochure with information on the project and a questionnaire. In the letter, brochure and appointmentflyer, a telephone number of the Public Health Service is provided where individuals could call for more information.

2.5 Questionnaires

There were three versions of the questionnaire, one for 0-11 year-olds (A), for 12-16 year-olds (B) and one for 17-79 year-olds (C). The questionnaire contained data on demographic variables (gender, marital status, date of birth, nationality (of parents), native country (of parents), number of persons in household, number of persons in household that visit a daycare center/elementary school), religion, vaccination (participation in the NIP, (necessity on) vaccination against DTP-IPV, MMR, Hib, vaccination against DTP, tetanus, hepatitis A, hepatitis B, influenza), travelling, longlasting coughing, recreation in fresh waters, sexual transmitted diseases, sexual history, contact with animals, self perception on health, chronic diseases, smoking and drinking habits, occupation and education.
Non-response questionnaires were also drawn up in three versions and covered age, country of birth, level of education, religion, participation in the NIP, opinion on vaccinations in the NIP and self-perception of health status.

The questionnaires were drawn up on the basis of the evaluation of the pilot-questionnaire and an inventory of wishes of researchers in the RIVM, working on infectious diseases. They were tested on readability and workability with colleagues and laymen. There are no versions of the questionnaires available in other languages. Persons interested can contact the authors for information.

2.6 Approaching the participants

One to one-and-a-half week before the consultation days in a particular municipality, a mailing package (invitation letter, a flyer with prescheduled appointment, a brochure with information on the project and a questionnaire with a individual number (U-number)) was sent to all eligible persons. The sampled individuals were asked to fill out the questionnaire at home and to visit the special Pienter clinic to give two tubes (2x10 cc) of blood.

Five to six days before consultation days in a municipality, all invited persons were approached by phone to remind them of the study, to answer possible questions and to ask if they were willing to participate. When individuals refused, they were asked to just fill in the questionnaire or in second instance answer some questions for the non-response survey (by telephone or mail).

When individuals were unable to come at the proposed time of appointment, they were offered an alternative: the walk-in consultation hours at night, the extra consultation hour the week after the regular hours or if necessary, a home-visit. Individuals who could not be reached by phone (not at home at several times, secret number, no telephone), were sent a written reminder.

Turkish and Moroccan persons were sent a translated letter in addition to the Dutch version and were not approached by phone but were paid a home-visit when not met at the consultation hour. Therefore a Turkish and a Moroccan (Arabian and Berber speaking) field work staff member were appointed; they could also give information to Turkish and Moroccan individuals in their own language at the consultation hours. These measures were taken to facilitate the response in these groups.

Persons who had not shown up at the consultation hours and had stated that they had intended to were approached again to invite them to the additional walk-in consultation hour one week later on Wednesday. Also individuals who could not be reached by phone before the regular consultation hours and who had not responded were approached again. Persons who refused to come to the extra consultation hour were asked to fill in the questionnaire or in second instance answer the non-response questionnaire (by phone or mail). Individuals who could not be

reached (not at home at several calls, secret number or no telephone) were sent the short non-response questionnaire.
Persons who had said they intended to visit the additonal walk-in consultation hour and had not shown up were sent the non-response questionnaire.

The approach of all eligible participants summarised :

	days before/after consultation hour
• written personal invitation with information	-10 days
• reminder by phone (or mail)	-4 days
• clinic	day 0
• non-response by phone (or mail)	+1 days
• additional clinic	+8 days
• non-response by mail	+9 days

2.7 Location of the consultation hours

The location for the blood sampling was arranged by the PHS. This could be at the PHS if located in the selected municipality or in any other appropriate building in that municipality. The location had to meet certain criteria:

- well-known location in the town/city
- sufficient parking space
- possibility for extra consultation hour at night (5.00 - 7.30 p.m.)
- separate waiting room and room for blood sampling
- refrigerator with enough room for temporary storage of approximately 300 blood tubes
- minimal two tables and six chairs
- if possible a telephone with respect to making appointments for home-visits and reachibility of the team
- if possible a sink and a lockable closet for gift vouchers en personal data

The name of the contact person of this location was given to the co-ordinator of the field work of the Pienter project.

2.8 Consultation hours

The consultation hours were planned weekly, with the exceptions of holidays, in the period October 1995 until December 1996. Appointments were made on Mondays and Tuesdays from 9.00 a.m. to 5.00 p.m. but individuals were allowed to come in until 7.30 p.m. at the walk-in consultation hour. The Wednesday of the following week an extra open consultation hour was planned from 5.00 p.m. to 7.30 p.m..

Participants were called in order of entry of the waiting room. First possible questions and remarks are answered. Then they were asked to sign an informed consent declaring that his/her serum will be tested for antibodies against several infectious diseases (except HIV), that he/she would receive no information on individual test results, that the collected data and sera would be anonymised after completion of the data collection in the municipality (i.e. after blood sampling) and that serum would be stored under a code for a long time for the purpose of public health research. When a participant was under twelve, one of the parents was asked to sign the informed consent. A field worker from the Pienter project also signed the informed consent showing that the investigators were committed to guarantee the items mentioned in the informed consent.

The questionnaire was checked on completeness. If necessary, missing or unclear answers were inquired about, except when it concerned a question on diagnosis of sexual transmittable diseases or on sexual history in order not to discomfort the participant. If a participant had been unable to fill in the questionnaire him/herself (e.g. a allochtonous person with insufficient knowledge of the Dutch language), it would be completed in co-operation with a staff member.

In case participants had brought a vaccination certificate -as was asked for-, data were written on a special study form and adhered to the questionnaire.
Stickers with the same personal numbers (U-numbers) as used in the invitation letter and questionnaire were sticked on the additional vaccination registration form; another personal number (S-number) was sticked on the same papers as well as on the two bloodtubes and on the serumtube. Both U- and S-numbers were unique for an individual. So every person in the study sample got a U-number allocated and only the participants who had given blood got an additional S-number. This way the questionnaire and data from the population registration (U-number) could be linked with the blood sample (S-number).

Generally, two tubes of 10 cc blood were drawn from each participant. With very young children where a vein punction was not possible, a heel prick or finger prick was done (1 cc blood).

Participants were offered a gift voucher of ƒ15,- as a token of gratitude and children also got funny little stickers.
At the consultation hours registration lists were available with names of invited individuals, U-numbers, date and time of the prescheduled appointment. This registration list could be used when a participant would come without any papers. Also stickers of U-numbers and S-numbers were available to adhere to the research materials.

A number of items on every participant were noted on a work list at the consultation hour or house-visit:

- personal 'invitation' number (U-number)
- personal 'serum' number (S-number)
- consultation hour or house-visit
- date consultation hour/house visit
- having signed informed consent
- bringing questionnaire
- bringing vaccination certificate
- number of tubes of blood taken
- gift voucher offered
- possible remarks (e.g. heel prick)

Per municipality the number of gift vouchers supplied and study materials used/to be ordered was registered.
Individuals who had not shown up at consultation hours, were again approached by phone or mail. They were asked to come to the extra walk-in consultation hour on the following Wednesday evening from 5.00 p.m. until 7.30 p.m. or in second instance, send in the questionnaire or non-response questionnaire. When individuals couldn't be reached or did not show up at the extra consultation hours, a non-response questionnaire was sent.

2.9 Serum extraction and storage

The blood samples collected at the consultation hours were put in the refrigerator during the day at the location of the consultation hour, transported in a cooler to the RIVM where they were stored in a refrigerator at night and processed the next day. The serum extraction was done with a TECAN pipette robot. The collected serum of a participant was divided in portions of 350 µml and stored in cups of 500 µl with stickers with the S-number (personal serum number) on them. The different portions are stored in different freezers at -86° Celsius.

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2.10 Vaccination certificates

If proof of previous vaccination could be derived from the vaccination certificates, dates of vaccination were written-down on a special registration form to be adhered to the questionnaire. This provided a check of self-reported vaccination history for those participants.

- NIP-certificate:

Diphtheria Pertussis Tetanus and Poliomyelitis (DTP-IPV), Diphtheria Pertussis and Tetanus (DTP), Mumps Measles and Rubella (MMR), Measles (M), *Haemophilus influenzaee* type b (Hib); other vaccinations could be noted in an extra category.

- Military service vaccination certificate:

Diphtheria Pertussis and Tetanus (DTP).

- Tropical travelling vaccination certificate:

Diphtheria Pertussis and Tetanus (DTP).

2.11 Complaint procedure

In the information brochure, invitation letter and appointmentflyer a phone-number of the PHS is mentioned for extra information, questions or remarks.

Complaints were expected to be expressed to:

- staff members of the PHS when called by the participants
- administrative staff members of the Pienter project when calling the participants
- field work team of the Pienter project at the consultation hours

Expected sort of possible complaints were:

- disagreement with design of the study, hesitations about anonymity, not getting notice on the laboratory results.
- disagreement with unsolicited mail/telephone call
- parking problems, long queues at the consultation hours and such like
- pain during/after blood donation, bruises after blood donation
- unfriendly treatment