Handbook of Endemic Treponematoses: Yaws, Endemic Syphilis, and Pinta

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Preface

In spite of a considerable decrease in the prevalence of yaws, endemic syphilis, and pinta as a result of WHO/UNICEF-sponsored national control campaigns during the 1950s and 1960s, these diseases are still endemic in many parts of the world. They are usually found in remote, rural populations that have little or no access to health care and among whom the large-scale treatment activities that are needed are the most difficult to apply. In addition, constant surveillance and active case-finding and case-reporting are essential to the success of control work; these are activities that may be performed by locally-based community health workers, with support and guidance from health services at the district and national levels.

This handbook is intended to be a reference source for health care workers and public health personnel throughout the tropical and subtropical world whose duties include the diagnosis, treatment, and prevention of yaws, endemic syphilis (bejel), and pinta. It is not a comprehensive essay on the biology of the treponemes and does not discuss the pathology of these diseases in detail. It describes briefly the clinical manifestations of each disease, supplementing each description with colour photographs of characteristic lesions. The treatment of the endemic treponematoses is described, with emphasis on the epidemiological methods used to control these diseases. This information should enable health workers to make a correct diagnosis, give the proper treatment, and control (or even eliminate) treponematoses in the population they serve.

Characteristics and history of the endemic treponematoses

Introduction

The endemic treponematoses—yaws, endemic syphilis (bejel), and pinta—are a group of chronic bacterial infections caused by treponemes. These organisms belong to the family Treponemataceae[1] and the genus *Treponema*. The agents of yaws, endemic syphilis, and pinta are *T. pertenue*, *T. pallidum*, and "*T. carateum*" (invalid)[2], respectively. Man is their only natural host.

Characteristics of treponemes

The treponemes that cause yaws, endemic syphilis, and pinta have identical morphology. Because of their small size and mass, they cannot be seen with an ordinary microscope unless a dark-field condenser is used. They look like thin, silver threads coiled like a corkscrew, and move with a characteristic rapid spinning motion.

The agent of venereal syphilis, also called *T. pallidum*, is identical in almost all respects to the organism that causes endemic syphilis. The difference is that late cardiovascular, neurological, and visceral complications are found with much greater frequency in venereal syphilis than in endemic syphilis. Thus, endemic syphilis is clinically similar to venereal syphilis, but epidemiologically is more closely related to yaws (Table 1).

There are a large number of non-pathogenic species of *Treponema* that are normally present in the mouth and intestinal and genitourinary tracts of man. These organisms are opportunistic pathogens and can stimulate the formation of antibodies that cross-react with the pathogenic treponemes of yaws, endemic syphilis, and pinta. While the avirulent treponemes can be cultured, the pathogenic treponemes do not grow *in*

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[1] The other members of the Treponemataceae are *Borrelia* and *Leptospira*, which cause relapsing fever and leptospirosis, respectively.
[2] Bacterial names appearing in quotation marks in the text have no standing in nomenclature since they have not been validated by the International Committee on Systematic Bacteriology.

Table 1. Epidemiological characteristics of treponemal diseases

Epidemiological characteristic	Treponemal disease			
	Venereal syphilis	Endemic syphilis	Yaws	Pinta
Occurrence	sporadic, urban	endemic, rural	endemic, rural	endemic, rural
Geographical distribution	worldwide	South-west Asia, sub-Saharan regions of Africa, Bosnia	Africa, south-east Asia, Western Pacific, South America, Caribbean	Central and South America, Mexico,
Climate in which the disease mostly occurs	all types	arid, warm	humid, warm	semi-arid, warm
Age group with peak incidence (years)	18–30	2–10	2–10	15–30
Transmissibility	high	high	high	low
Mode of transmission: Direct (person to person)				
Sexual	usual	no	no	no
Non-sexual	rare	yes	usual	probable
Indirect				
Utensils	rare	usual	rare	unknown
Contaminated fingers	unknown	unknown	probably frequent	unknown
Congenital	occasional	unknown	no	no
Reservoir of infection	adults	children 2–15 years old; contacts in home, school and village; latent cases capable of becoming active	children 2–15 years old; contacts in home, school and village; latent cases capable of becoming active	cases with long-standing skin lesions

vitro. *T. pallidum* and *T. pertenue* are maintained in the laboratory by infecting laboratory animals or by freezing the organisms (at $-70\,°C$ or below) in infected tissue or in special solutions.

The treponemes of yaws, pinta, and the different types of syphilis are closely related. Infection with one organism provides partial protection against infection by another, which indicates that they share common antigens. There is no laboratory test that can distinguish these treponemes from one another. *T. pallidum* and *T. pertenue* cause different lesions when inoculated into rabbits and hamsters, but this has no practical significance. "*T. carateum*", on the other hand, produces lesions only in man and higher apes.

The most notable characteristic of the pathogenic treponemes is their tendency to cause a chronic infection that progresses by stages of

clinically apparent disease. Two stages are usually recognized: early and late. Each stage may present lesions that differ in location and morphology. In yaws and endemic syphilis, only the lesions of the early stage are infectious, and may recrudesce during the first 5 years of latency and serve as a source for new cases in the community.

The close relationship between the pathogenic treponemes suggests that they have a common ancestor. A popular theory speculates that a single treponemal disease originated in primitive man in equatorial Africa. It was transmitted by social contact, and over the millennia, it spread the world over, following man's migrations. This disease changed its characteristics according to place, race and climate, giving rise first to pinta and then to yaws and endemic syphilis. According to this "unitarian" theory, a mutation caused the organism of endemic syphilis to become more virulent, which led to the appearance of venereal syphilis in Europe in the late 15th century.

Whatever their origin, the endemic treponematoses were until recently among mankind's most common afflictions. In the land mass between the Tropics of Cancer and Capricorn, these infections constituted a vast public health problem. Although seldom, if ever, fatal, the treponematoses caused serious public health, social and economic problems in the communities in which they occurred.

History of epidemiology and control programmes

The first effective drugs for the treatment of yaws and venereal and endemic syphilis were the arsenicals, which were discovered by Dr Paul Ehrlich in 1910. They remained the drugs of choice until penicillin became widely available in the 1940s. Unfortunately, these drugs had serious toxic side-effects. It was necessary to give arsenicals by a series of injections over a period of several weeks to achieve a cure. In some areas the prevalence of clinical yaws did decrease after treatment of all active cases with arsenicals and other metal therapy. However, this did not eliminate yaws from the population because it was not then appreciated that treatment was also necessary for the patient's symptomless contacts who had incubating or latent infections.

The remarkable curative power of benzylpenicillin (penicillin G) in venereal syphilis was demonstrated in 1943, and shortly thereafter in yaws, endemic syphilis, and pinta. The need to give several injections of benzylpenicillin as well as its high cost limited the use of the drug for the treatment of the endemic treponematoses until the late 1940s, when improved technology lowered manufacturing costs and led to the development of inexpensive, long-acting penicillin preparations. These long-acting, repository benzylpenicillin preparations—namely, penicillin aluminium monostearate (PAM) and benzathine benzylpenicillin—were very effective against the endemic treponematoses when given in a single intramuscular injection.

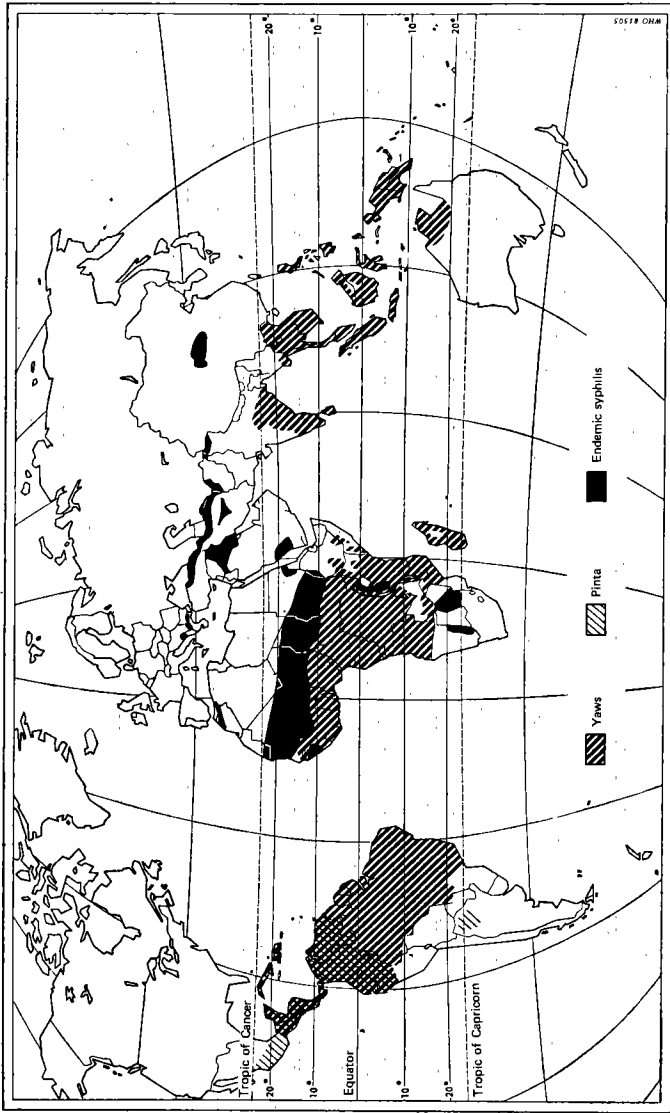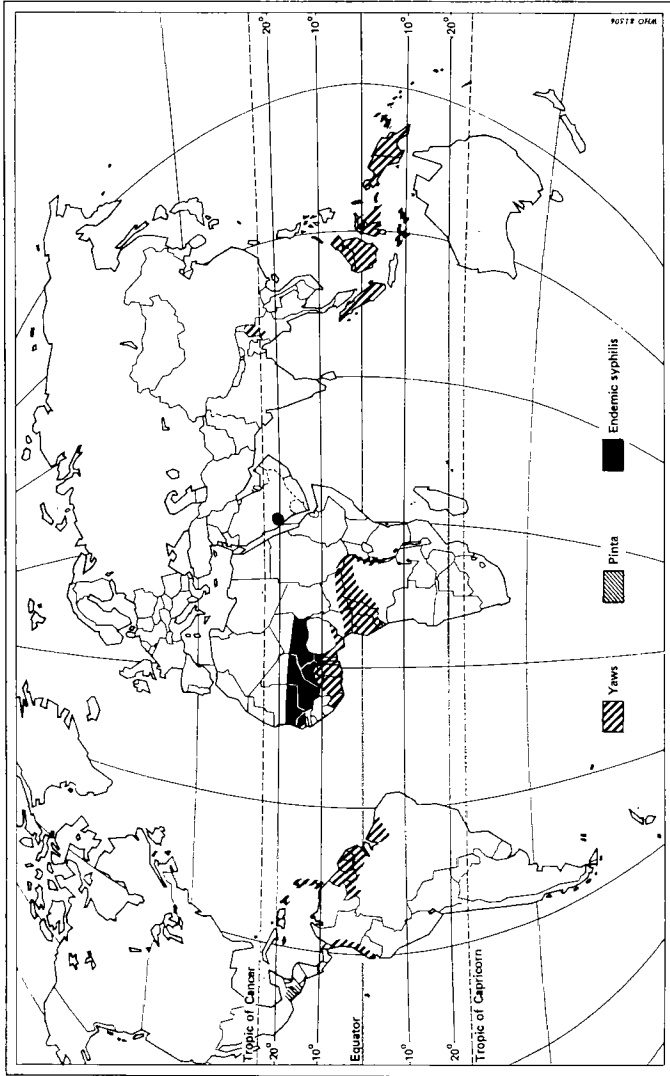Fig. 1. Geographical distribution of the endemic treponematoses in the early 1950s

Fig. 2. Geographical distribution of the endemic treponematoses in the early 1980s

Yaws control campaigns using PAM or benzathine benzylpenicillin in Haiti, Indonesia and Jamaica were remarkably successful in lowering the prevalence of the active form of the disease. These campaigns also established the epidemiological concept that penicillin treatment was necessary for asymptomatic household contacts and presumed latent cases, in order to abolish the reservoir of infection.

In 1948, WHO, together with UNICEF, established a global yaws control programme based on the premise that mass penicillin treatment, if carefully planned and carried out, would result in a significant reduction in the incidence of infectious cases, which could then be kept to a minimum by thorough case-finding and preventive measures. Because yaws is so closely related clinically and epidemiologically to endemic syphilis, and to a lesser extent to pinta, mass penicillin treatment was later extended to include these infections.

Mobile teams were formed to give penicillin treatment to yaws patients and their contacts. Where the prevalence of cases with active lesions was 10% or greater, penicillin was given to the entire community; where the prevalence was between 5 and 10%, penicillin was given to patients, household contacts, and all children under 15 years of age; where the prevalence was less than 5%, only active cases and household contacts were treated. Follow-up surveys of treated communities were deemed essential in order to prevent reinfection of the community and to detect and treat: (a) cases that may have been missed; (b) those in whom the treatment had failed; and (c) infected immigrants. The case-finding techniques used in these surveys included house-to-house searches by "yaws scouts"—a technique later used with great success in the smallpox eradication programme. Mass campaigns were organized and coordinated to create ever-enlarging yaws-free areas. Every effort was made to integrate post-campaign yaws surveillance into the permanent health service of the community where such existed. Experienced mobile teams were assigned to groups of health posts to follow up all new active cases of the disease.

The problems facing the yaws control programmes were enormous. About half of the 400 million people living in the tropical belt between the Tropics of Cancer and Capricorn were likely to be exposed to yaws during their lifetime (Fig. 1). Most of these people lived in the warm, humid, rural parts of Africa, America, south-east Asia, Australia and the neighbouring Pacific islands, and the Indian subcontinent. Up to 80% of those exposed were infected, and at any given time, up to 20% of those infected had clinical yaws. Approximately 10% of those infected with yaws were invalids because of late crippling lesions or were severely disfigured.

Foci of endemic syphilis were present in Afghanistan, North Africa, southern Africa, south-west Asia, China and Europe. The largest concentration of cases was in south-west Asia and the sub-Saharan regions of Africa. The estimated prevalence of clinical disease ranged from 3% to 5% of the population, but the incidence of late destructive lesions was much higher than that found in yaws.

Pinta was confined to the western hemisphere, and in the 1950s, there were an estimated one million cases in Central America, northern South America, and Mexico.

In the 1960s, in many countries the mobile teams that conducted the yaws treatment campaigns were dismantled or given other assignments and, although an effort was made to incorporate yaws control into the primary health care system, there was little active case-finding or prophylactic treatment of contacts. This led to the persistence or resurgence of endemic foci, from where the infection is again spreading, thus threatening the gains made by previous mass-treatment campaigns. This applies particularly to areas of western and central Africa and, to a lesser extent, to Asia.

Over the past 10 years, the trends in the incidence of the endemic treponematoses have differed according to the particular disease and the geographical region. Pinta continues to decrease in prevalence and is at present restricted to a few areas of Central America, Colombia, and southern Mexico, although only limited surveillance has been done in the past decade (Fig. 2).

Endemic syphilis has also decreased in prevalence in the world as a whole, but foci of infection persist in Africa and south-west Asia. A recent survey found thousands of cases of early endemic syphilis in Mali, Mauritania, Niger, and Upper Volta. This suggests that today endemic syphilis may be a much greater problem in sub-Saharan Africa (Sahel) than it was formerly.

Yaws has shown the greatest changes in regional prevalence since the mass treatment campaigns. In South America only scattered foci of active yaws persist. Previously heavily infected countries such as Brazil and Suriname are almost yaws-free, and in other areas such as Colombia, Ecuador, French Guiana, and Guyana only a few dozen or hundred cases are reported annually. However, there is little active case-finding in these countries. In south-east Asia, yaws still exists in Indonesia and Papua New Guinea.

Africa remains the main reservoir of yaws in the world. Several patterns of yaws prevalence are evident. In many countries, e.g., Ivory Coast and Nigeria, clinical cases of yaws are declining owing to a combination of improved rural health care and improved standards of living. In other countries, yaws has increased to levels approaching those of the pre-campaign era. An example is Ghana, where premature curtailment of surveillance by mobile medical field units and economic difficulties have contributed to the recrudescence of the disease.

Yaws is underreported in most African countries since the disease occurs predominantly in remote rural areas or among isolated tribes, such as the Pygmies in the Central African Republic, the Republic of Cameroon, and Zaire. Finally, countries that have achieved good control over endemic treponematoses are constantly under the threat of importation of these diseases from nearby areas. Without renewed control programmes, the gains made by the mass treatment campaigns of 20 years ago will soon be lost in some African countries.

Yaws

In areas where yaws has long been endemic, there are names for it in the local language or dialect. Some of its synonyms are: *pian* (French); *framboesia* (German, Dutch); *buba* (Spanish); *bouba* (Portuguese).

Causative agent

The organism responsible for yaws is *Treponema pertenue*. It is identical in appearance to *T. pallidum* (the organism that causes venereal and endemic syphilis) and "*T. carateum*" (the cause of pinta). *T. pertenue* does not cause congenital infections because it cannot cross the placenta. It produces lesions in the skin, bone, and cartilage, but not in deeper tissues or organs. Like other pathogenic treponemes, it is easily killed by drying, exposure to oxygen, and elevated temperature. The organism multiplies very slowly (once every 30–33 hours) in man and experimentally infected animals. It does not grow in culture.

Occurrence

Yaws occurs primarily in the warm, humid, tropical areas of Africa, Central and South America, the Caribbean and the equatorial islands of south-east Asia. In the endemic areas where wet and dry seasons alternate, clinical manifestations and the prevalence of infectious yaws lesions increase during the rainy season.

Reservoir

Children aged 2–15 years and latent cases serve as the reservoir of infection. A yaws-like treponeme (the so-called "*T. fribourg–blanc*" (invalid)) has been isolated from west African monkeys and baboons, but its significance for human yaws is unknown.

Mode of transmission

Yaws is transmitted by direct (person-to-person) non-sexual contact with the exudate or serum from infectious yaws lesions (early or relapse papules, papillomata, ulceropapillomata, or macules). Late yaws lesions (deep ulcers, gangosa, bone, and hyperkeratotic palmar and plantar lesions) are not infectious. Indirect transmission by insects and contaminated utensils (fomites) is generally of limited significance.

The spread of yaws may be facilitated by crowding and poor community sanitation. The lack of water and soap for bathing and washing and of shoes and clothing for children between the ages of 5 and 15 years are said to favour yaws transmission.

Course of infection

The clinical course of a hypothetical case of yaws is as follows. The initial or primary papule, sometimes called the mother yaw, appears on the skin at the site of entry of *T. pertenue* after an incubation period of 9–90 days (average, 21 days). The site of entry is often a pre-existing abrasion, laceration or insect bite. During the incubation period the organism multiplies at the infection site, invades subcutaneous lymphatics, and spreads through the bloodstream.

The yaws papule enlarges to become an early papilloma or framboesioma; it is very rich in treponemes. This lesion usually lasts 3–6 months. It may heal spontaneously before the appearance of the first crop of early secondary yaws lesions, thereby creating a brief period of latency.

The early secondary yaws lesions may appear on the skin near the initial lesion or elsewhere in the body, including bone and cartilage. These lesions result from autoinoculation and from the spread of *T. pertenue* systemically. Each crop of early secondary lesions may persist for more than 6 months; the lesions heal spontaneously, and do not leave scars unless they become ulcerated and secondarily infected by certain other bacteria. The disease then enters a non-infectious latent period, which may last the lifetime of the patient.

The state of latency can be interrupted at any time by the reappearance or relapse of infectious yaws lesions. These relapses tend to occur at intervals for up to 5 years after infection. Relapsing lesions tend to be localized to the periaxillary, perianal, or circumoral areas. The total duration of infectiousness for an untreated yaws patient, including relapses, is probably of the order of 12–18 months.

Late, active yaws lesions are often destructive and develop in as many as 10% of cases; these lesions may develop early in the course of the infection, but they more usually appear several years after the initial infection.

Types of yaws lesion

Yaws produces a great variety of skin, bone and joint lesions (the terms used to describe or modify the description of skin lesions are defined in the glossary presented in Annex 1). The cutaneous yaws lesions have a number of common characteristics:

— Early lesions are often pruritic, and scratching facilitates both spread of the infection to other areas of the body by autoinoculation and the transmission of the disease within the community.
— The early lesions tend to occur in crops, which often overlap with one another.
— Mixed (polymorphous) forms of lesions are often present in the same patient;
— A change in climate may influence the number and morphology of yaws lesions. In the dry season fewer lesions are present and they tend to be of the macular type; papillomata tend to retreat to the more humid areas of the body surface such as axillae and anal folds.
— Induration is not a common feature of early yaws lesions.

Despite the variety of yaws lesions, in endemic areas the disease can usually be accurately diagnosed on the basis of clinical findings alone.
Constitutional symptoms such as fever and malaise are not significant in yaws. The lymph nodes draining cutaneous lesions are frequently enlarged and tender, but they do not suppurate. Nocturnal bone pain and tenderness of the tibial shaft and other long bones due to periostitis are common in early yaws.
The nomenclature and classification of yaws lesions are given in Table 2.

Differential diagnosis

Diseases commonly confused with yaws are as follows:

— *Impetigo.* A common skin infection of children caused by streptococci or staphylococci (Fig. 40 and 41).
— *Tinea versicolor* (pityriasis versicolor). A superficial skin infection caused by the fungus, *Malassezia furfur,* characterized by fawn-coloured scaling macules or patches on shoulders, chest, upper back and abdomen (Fig. 42).
— *Molluscum contagiosum.* A viral disease of the skin producing pink or white papules with a prominent central core, which may appear anywhere on the body (Fig. 43).
— *Scabies.* Infestation of human skin by *Sarcoptes scabiei,* producing cutaneous papules or vesicles caused by the burrowing into the skin of the mite. Lesions are prominent around finger webs and the anterior surfaces of elbows and wrists. Scabies is frequently accompanied by severe itching (Fig. 44 and 45).

Table 2. Classification of yaws lesions

Early yaws lesions	Examples	Infectiousness[a]
Initial lesions	papilloma (Fig. 3)	+ + +
Papillomata	papillomata (Fig. 4, 5, 6, 7, 8, 9, 10) serpiginous papilloma (Fig. 11) ulceropapillomata (Fig. 12, 13, 14)	+ + +
Macules	squamous macules (Fig. 15 and 16) palmar (Fig. 17), plantar (Fig. 18)	+
Maculopapules	maculopapulomatous (Fig. 19) mucocutaneous (Fig. 20)	+ +
Papules	squamous micropapules (Fig. 21)	+ +
Micropapules	polymorphous (Fig. 22)	+ +
Nodules	Fig. 23	+
Plaques	Fig. 24	+
Hyperkeratosis	plantar (Fig. 25, 26, 27) palmar (Fig. 28)	−
Bone and joint lesions	polydactylitis (Fig. 29) osteoperiostitis (Fig. 30 and 31)	− −
Late yaws lesions		
Hyperkeratosis	Fig. 26, 27, 28 (hyperkeratotic lesions may be similar in both late and early yaws)	−
Nodular	scars (Fig. 32)	−
Ulcerated nodular	gangosa (Fig. 33, 34, 35);	−
Plaques	osteoperiostitis (Fig. 36);	−
Bone and joint	sabre tibia (Fig. 37); gondou (Fig. 38); monodactylitis (Fig. 36)	−
Juxta-articular nodules	Fig. 39	−

[a] − = not infectious; + = infectious; + + = very infectious; and + + + = highly infectious.

— *Lichen planus.* A chronic inflammatory disease of unknown etiology characterized by flat-topped, shiny papules with a characteristic violet hue (Fig. 46).
— *Tropical ulcer* (ulcus tropicum). A painful ulcer that usually occurs on the lower limbs in the humid tropics. It is caused by a mixed infection with "*Treponema vincentii*", *Fusobacterium nucleatum* and other bacteria. In contrast to yaws ulcerations, tropical ulcers have well-defined edges, a purulent base, and may penetrate into tendons and bone (Fig. 47).
— *Plantar warts* (verruca plantaris). A tender, flat wart on the sole of the foot caused by a papovavirus; may be confused with plantar papilloma (Fig. 48).
— *Tungiasis* (jiggers). Plantar lesions caused by the burrowing of the female sand-flea, *Tunga penetrans* (Fig. 49).
— *Cutaneous leishmaniasis.* An indurated, usually solitary nodule or chronic ulceration caused by *Leishmania* species (Fig. 50).

— *Leprosy*. The lesions of both lepromatous and tuberculoid forms of leprosy caused by *Mycobacterium leprae* may be mistaken for yaws; however, anaesthesia is never caused by yaws (Fig. 51).
— *Psoriasis*. This chronic, hereditary skin disease may sometimes be mistaken for yaws. Its distinctive lesions are red macules covered almost to their edges by whitish or silvery lamellated scales. It usually involves the knees, elbows, trunk, and scalp (Fig. 52 and 53).

Endemic syphilis

Some of the common synonyms of endemic syphilis are: *bejel* (Arabic), *njovera, dichuchwa* (in Zimbabwe), endemic syphilis of Bosnia, and non-venereal or childhood syphilis. Extinct forms of the disease are believed to include the sibbens of Scotland in the 17th century, the *radesyge* of Norway in the 18th century, and the *skerljevo* of the Croatian Coast (Yugoslavia) in the 19th century.

Causative agent

Endemic syphilis is caused by *Treponema pallidum*. This organism is closely related, if not identical, to the *T. pallidum* of venereal syphilis. Many believe that the subtle antigenic and pathogenic differences between endemic and venereal syphilis represent only strain variations of the same organism. Endemic syphilis acquired in childhood protects against later infection with venereal syphilis.

Like yaws, endemic syphilis is a chronic, childhood infection of skin, bone and cartilage. Some lesions of endemic syphilis are indistinguishable from those of yaws (e.g., gangosa, osteoperiostitis) or venereal syphilis (e.g., squamous, macular, palmar, and plantar syphilis, mucous patches). In contrast to venereal syphilis, however, primary lesions, congenital infections, and late neurological and cardiovascular complications occur rarely, if ever, in endemic syphilis (Table 3). When these symptoms have been observed in areas with endemic syphilis, it has been impossible to exclude the sporadic occurrence of venereal syphilis in the same geographical area.

Occurrence

Once highly prevalent in the nomadic and seminomadic rural populations of parts of north Africa, south-west Asia, and the eastern Mediterranean basin, endemic syphilis is prevalent today primarily among the seminomads in the Arabian peninsula and along the southern border of the Sahara desert in Africa (the Sahel). It was never prevalent

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Table 3. Clinical characteristics of treponemal diseases

Clinical characteristics	Treponemal disease			
	Venereal syphilis	Endemic syphilis	Yaws	Pinta
Initial lesion	common	rare	common	common
Location	genitals	oral mucosa	extremities	extremities
Disseminated lesions	80–100% of cases	90–100% of cases	90–100% of cases	common
Location	systemic	intertriginous areas	skin, bone	local
Extent	widespread	limited	widespread	limited
Constitutional symptoms	common	rare	rare	never
Regional lymphadenopathy	common	common	common	infrequent
Infectious relapses	25% of cases	unknown	75–90% of cases	none
Late complications:				
Estimated frequency	35% of cases	frequent	10% of cases	very frequent
Gummata/ulcers	10–15% of cases	25–50% of cases	10% of cases	rare
Location	skin, bone, viscera	bone	bone	skin
Neurological	10% of cases	unknown	never	never
Cardiovascular	10–15% of cases	unknown	never	never

in the western hemisphere, and some scattered endemic foci of endemic syphilis in central Asia, Australia and India were treated and eliminated by penicillin treatment campaigns in the 1950s.

In contrast to yaws, endemic syphilis is prevalent in dry, arid climates. A higher proportion of the population is likely to be infected with endemic syphilis than is the case with yaws in endemic areas.

Reservoir

Children aged 2–15 years with early infections, as well as latent cases of the disease, serve as the reservoir of infection.

Modes of transmission

Because the most common initial lesion of endemic syphilis occurs on the oral mucosa, indirect transmission of the disease via contaminated drinking vessels is thought to be the most common mode of

transmission. Direct lesion-to-skin contact among children and contact with fingers contaminated with saliva containing treponemes are also important transmission mechanisms. The disease tends to occur in family groups, the infection being acquired first by children who spread it to susceptible adults.

Another factor that may facilitate transmission is angular stomatitis, which may be caused by riboflavin deficiency. Flies may also be important vectors transmitting the disease.

Course of infection

A primary lesion is rarely seen in endemic syphilis. The first lesions to appear are mucous patches on the oropharyngeal mucosa, which may be followed by a variety of secondary-type rashes or lesions. The latter prefer the moist body surfaces such as the axillary and genital areas.

The early disease may be followed by a latent period of variable duration. It is not known if infectious relapses occur.

Most patients develop some lesion of late endemic syphilis such as a granulomatous ulcer or nodule. The most common are rhinopharyngitis mutilans (gangosa) and osteoperiostitis. These complications disable and deform the individual, but they are seldom a cause of death.

Types of lesion

The most common early lesions, in decreasing order of frequency, are:

— *Mucous patches*. These are shallow, relatively painless ulcerations located on the pillars of the fauces, tonsils, tongue, lips, and buccal mucosa; they are frequently accompanied by hoarseness due to syphilitic laryngitis (Fig. 54).

— *Angular stomatitis or split papules*. These are also found in early yaws (Fig. 55).

— *Osteoperiostitis*. A common early manifestation of endemic syphilis, which usually involves the long bones of the lower extremities, causing nocturnal leg pains. It is similar in all respects to osteoperiostitis of yaws (Fig. 30 and 31).

— *Disseminated papules*. These papular eruptions do not itch; they also occur in venereal syphilis (Fig. 56).

— *Condylomata*. These raised, indurated lesions are very similar to yaws papillomata and tend to occur in moist areas of the skin (Fig. 57).

Other early lesions include macular, papular, papulosquamous, annular, and circinate (coin-like) lesions, to name a few.

In late endemic syphilis, gummata of the nasopharynx, skin and bone are common. These may progress to destructive chronic ulcers (Fig. 58), producing gangosa-like lesions (Fig. 59).

Differential diagnosis

The same diseases that confuse the diagnosis of yaws and venereal syphilis must be taken into consideration when a sporadic case of endemic syphilis is being evaluated. It should be borne in mind that venereal syphilis can also be transmitted by non-venereal, person-to-person contact among children.

Pinta

·Common synonyms of pinta include, *mal de pinto* (in Mexico), *carate* (in Colombia and Venezuela), *azul* (in Chile and Peru).

Causative agent

"*Treponema carateum*" is the organism responsible for pinta. The disease was thought to be a fungal infection until 1938, when serous exudate from a lesion in a Cuban patient was shown to contain treponemes indistinguishable from those causing yaws and syphilis. "*T. careteum*" is pathogenic only in man and the higher apes, and it provides some cross-immunity to yaws and syphilis.

Occurrence

Pinta is thought to be a very ancient disease found only in the western hemisphere. Formerly, it was highly prevalent in the semi-arid regions of Brazil, Colombia, Cuba, southern Mexico, and Venezuela, with scattered foci in other Central and South American countries and the Caribbean islands; but today only scattered foci remain in northern South America and Mexico.

Like yaws and endemic syphilis, pinta is found in remote, rural communities. It differs from yaws and endemic syphilis in that it affects children and adults of all ages.

Reservoir

The main reservoir of pinta is thought to be young adults of 15–30 years of age who have skin lesions of long duration.

Mode of transmission

The precise mode of transmission is not known, but repeated direct, lesion-to-skin contact is the likely mechanism. Treponemes are abundant

in early lesions and persist through to the late dyschromic stage, which may be reached up to 40 years after the infection.

Course of infection

The usual incubation period is 2–3 weeks. The initial lesion is a papule or an erythematosquamous plaque. It is almost always located on an uncovered part of the body, usually the legs, the dorsum of the foot, the forearm, or the back of the hands.

The papule (or plaque) enlarges slowly by local extension or by merging with satellite lesions to form a hyperkeratotic, pigmented lesion accompanied by an enlargement of the lymph nodes draining the lesion.

Disseminated lesions identical to the initial lesions develop 3–9 months after infection. These "pintids" vary in number and location. They may slowly enlarge and merge to reach a diameter of 7–25 mm. The lesions become pigmented with age, changing slowly from a copper colour to lead-grey to slate-blue as a result of photosensitization.

Late pinta is characterized by pigmentary changes, from dyschromic treponeme-containing lesions to achromic treponeme-free lesions. This depigmentation process occurs at different rates even within the same lesion, giving rise to different degrees of hypochromia and atrophy around dyschromic and achromic lesions. No disability or complication other than leukoderma occurs.

Types of lesion

The types of lesion seen in pinta are as follows:

— erythematosquamous plaque (Fig. 60);
— violaceous psoriatic plaque (Fig. 61);
— late pigmented pinta, blue variety (Fig. 62);
— hyperpigmented, atrophic skin of late pinta (Fig. 63); and
— achromic scars of late pinta (Fig. 64).

Differential diagnosis

Early pinta may be difficult to distinguish from neurodermatitis, psoriasis and tinea versicolor. The leukoderma of late pinta may resemble vitiligo, and the late scars of yaws.

Venereal syphilis

A brief description of venereal syphilis is included here because some of its lesions are identical to those found in yaws and endemic syphilis. Venereal syphilis should always be considered when diagnosing sporadic cases of early yaws or endemic syphilis in sexually active individuals.

Causative agent

Venereal syphilis is caused by *Treponema pallidum*, which is morphologically identical to other pathogenic treponemes. Different strains of the organism or the age of the patient at the time of infection may account for the differences between endemic and venereal syphilis.

Occurrence

The disease occurs throughout the world, increasingly in former yaws endemic areas. The cases tend to be sporadic and concentrated in urban areas. The majority of cases occur in sexually active adults between 15 and 30 years of age.

Reservoir

Sexually active adults of both sexes who have active or latent infections of up to 2 years' duration, especially prostitutes, soldiers, sailors, and homosexual men, constitute the reservoir of infection.

Mode of transmission

Transmission occurs usually by direct contact during sexual intercourse or by close physical contact with infectious cases (see Table 1). Venereal syphilis in prepubescent children is usually acquired by sexual contact with infected adults, although transmission by non-venereal contact among children has been documented.

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Like other pathogenic treponemes, *T. pallidum* cannot penetrate intact skin. It enters the body through the mucous membranes or through small abrasions or lacerations. The organism enters the bloodstream soon after invasion and spreads to all organs and tissues during the incubation period. It appears in the blood intermittently throughout the course of the disease, which explains why venereal syphilis can be transmitted by a mother to her fetus or by blood transfusion.

Course of infection

Three distinct stages of infection are recognized: primary, secondary and tertiary. Usually each stage is separated by a latent period during which there are no visible signs of infection.

The primary lesion—the chancre—appears at the site of entry after an incubation period ranging from 9 to 90 days (average 3 weeks). It is a small, painless, genital papule or a shallow ulcer with indurated edges. Chancres on the penis and vulva are usually accompanied by moderate enlargement of the inguinal lymph nodes. The chancre heals spontaneously over a period of 2–6 weeks, and may be followed by a brief latent period.

The secondary stage is characterized by the appearance of disseminated lesions on the skin and in the internal organs several weeks after the chancre heals, or 6 weeks to 6 months after the initial infection. In women, these lesions are often the first overt sign of infection.

Secondary lesions vary greatly in appearance and location. In general, the lesions do not itch, are not painful, and seldom appear as blisters or vesicles. In contrast to yaws and endemic syphilis, secondary lesions are routinely accompanied by fever, malaise and generalized enlargement of the lymph nodes. The lesions heal after several weeks without leaving scars.

Another latent period lasting between 1 and 20 years, or even longer, follows the secondary stage. About 25% of untreated patients will experience a relapse of secondary lesions during the first 2 years of infection.

The late or tertiary stage develops in about one-third of untreated cases after a latent period of a few years to several decades duration. The tertiary lesions may involve the heart and blood vessels (cardiovascular syphilis), causing dilatation of the aortic valve and aneurysm of the thoracic aorta. The involvement of the central nervous system (neurosyphilis) causes a form of insanity (general paresis) or a loss of positional sense and sensation (tabes dorsalis). Gummata, a benign manifestation of tertiary syphilis, can occur in any organ or tissue of the body. Those involving skin and bone are indistinguishable from those found in yaws and endemic syphilis.

Congenital syphilis occurs when *T. pallidum* circulating in the infected mother's bloodstream crosses the placenta and enters the bloodstream and tissues of the fetus. This may cause the fetus to be stillborn or born

prematurely. Secondary-type lesions are present at birth or develop within the first 6 months of life.

Types of lesion

The types of lesion seen in venereal syphilis are as follows:

— *Chancre*. This lesion appears on the genitalia, and in women it is usually hidden on the cervix or the vaginal wall. It is a shallow ulcer with elevated, indurated (hardened) borders. It is painless and does not itch (Fig. 65).
— *Secondary papular rash*. This usually does not itch and may appear on the palms and soles (Fig. 66).
— *Secondary annular rash*. This circinate (coin-like) rash on the face is characteristic of venereal syphilis (Fig. 67).
— *Condylomata lata of secondary syphilis*. These lesions are similar to those of yaws (Fig. 68).

Diagnosis of the treponematoses

A presumptive diagnosis of yaws, pinta, or endemic syphilis can usually be made by careful assessment of the clinical manifestations together with the epidemiological features that characterize the infections (Tables 1 and 2). Early yaws and endemic syphilis are easily differentiated from pinta, and to a lesser extent, from venereal syphilis. The differentiation between sporadic cases of yaws and endemic syphilis, however, may be impossible. These diseases may coexist in certain geographical areas and their clinical manifestations are often indistinguishable. In such circumstances the diagnosis is usually based on a carefully taken case history and on epidemiological considerations.

Venereal syphilis should always be suspected in any sexually active person who presents with yaws-like lesions and who lives in a community where yaws and endemic syphilis are rare or absent. Migration of young adults of both sexes from rural to urban areas in search of work has led to a dramatic increase in the prevalence of venereal syphilis and other sexually-transmitted diseases in the tropical and subtropical world.

Laboratory diagnosis

Because the pathogenic treponemes cannot be grown in culture, the only practical way to identify these organisms in infected tissue is by microscopic examination of lesion secretions or by histopathological examination of biopsy tissue stained by special methods. The easiest, most sensitive and specific test is to identify freshly isolated, viable treponemes using the dark-field microscope technique. Serological tests to detect treponemal antibodies are also used as case-finding tools and to support a clinical diagnosis of a treponematosis, but they are not as specific as the dark-field examination. These tests will be discussed individually.

Dark-field examination technique

The pathogenic treponemes are 0.3 μm wide and 6–20 μm long, and are too small to be seen with an ordinary light microscope. In order to

see the organism, a special substage dark-field condenser must be used in place of the normal Abbé condenser, and the objective lens must have a built-in adjustable diaphragm or a funnel stop.

The lesion to be examined[1] is cleaned thoroughly with swabs soaked in saline, and blotted until any gross bleeding subsides. The clear serous exudate from the lesion is collected by touching the surface of the lesion with a microscope glass coverslip so that a drop of exudate adheres to it. The coverslip is then placed on a thin glass microscope slide and the specimen is spread thinly between the two surfaces. Alternatively, the specimen can be collected from the lesion surface, using a sterile large-bore needle and syringe, or from an enlarged regional lymph node after first injecting 0.5–1 ml of sterile saline.

Under the dark-field microscope, cells, bacteria, and debris appear as brilliant white objects on a dark background. Freshly isolated pathogenic treponemes appear as thin silver threads with a length of 1–3 times the diameter of an erythrocyte. They have several regular coils or spirals (length 1.5 μm) tightly wound along their length (Fig. 69). Their most characteristic feature is a rapid, corkscrew-like rotation which is frequently interrupted by thrashing or flexing movements from which they snap back to their original coiled form in a spring-like manner. The two ends of the organism are pointed.

A number of avirulent species of *Treponema* ("*T. microdentium*", "*T. macrodentium*") are normally present in the microbial flora of the mouth and may contaminate the specimens taken from oral mucous membrane lesions. These organisms must not be mistaken for the pathogenic *T. pallidum* or *T. pertenue*. Avirulent treponemes have fewer and more irregular spirals than do the pathogenic treponemes. These organisms may contaminate or be opportunistic invaders of any lesion in the anogenital area.

Lesion biopsy

Some treponemal lesions contain few organisms, particularly the late lesions of skin and bone. The dark-field examination in these cases is usually negative, while a biopsy may show characteristic histopathological changes or reveal treponemes when the specimen is stained by silver impregnation techniques (Fig. 70). These techniques, however, are less specific than is the dark-field examination, and are far more expensive. Moreover, they are not easily carried out in the field setting.

The tissue chosen for biopsy should include a margin of normal tissue, and be fixed in phosphate-buffered saline (pH 7·2) containing 100 ml of formalin per litre. The specimen should be marked with a label indicating the source, patient's name, and suspected disease before it is sent to a reference laboratory.

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[1] Protective rubber gloves must be worn during examination.

Serological tests

The host responds to the invasion by treponemes and to their
multiplication by producing a variety of antibodies and immune
cells. This immune response begins shortly after infection and peaks
towards the end of the secondary stage of generalized lesions—a time
when most of the organisms succumb to the immune defence
mechanisms.

The serological tests used in yaws, endemic syphilis, and pinta were
originally designed for use in venereal syphilis. These tests are divided
into two categories based on the type of antigen used (Table 4).

Table 4. Serological tests for syphilis, yaws, and pinta

Category	Nature of test	Antigen used	Sensitivity	Specificity	Types of test
Nontreponemal antigen	Flocculation	Cardiolipin	Good	A number of other diseases give false-positive reactions	VDRL (Venereal Disease Research Laboratory); RPR (rapid plasma reagin)
Treponemal antigen	Indirect fluorescent antibody	Lyophilized *T. pallidum*	Good	Very good	FTA-ABS (fluorescent treponemal antibody-absorption)
	Haemagglutination	Lysate *T. pallidum* attached to sheep or turkey erythrocytes	Less sensitive than FTA-ABS in primary syphilis	Very good	TPHA (*T. pallidum* haemagglutination assay); MHA-TP (micro-haemagglutination assay—*T. pallidum*)

Nontreponemal antigen tests are conducted with a complex phos-
pholipid antigen, cardiolipin, which is commercially extracted from
bovine heart. Tests in this category are also referred to as reagin tests,
nonspecific antibody tests or by the general term, serological tests for
syphilis (STS). The second category comprises treponemal antigen tests.
These use live or killed *T. pallidum*, or a treponemal group antigen
prepared from the non-pathogenic Reiter strain of "*T. phagedenis*"
(invalid). They are sometimes called specific antibody tests or
treponemal antibody tests or confirmatory tests. None of these tests
differentiates between the different pathogenic treponemes; they only
indicate a current or past infection with one or more of these organisms.

Commonly used *nontreponemal antigen tests* are the Venereal Disease Research Laboratory (VDRL) test, developed by the Public Health Service of the USA, and the rapid plasma reagin (RPR) test. The reagents and control sera needed to perform these tests are standardized and are commercially available throughout the world. Slightly modified versions of these tests are used for testing cerebrospinal fluid, unheated serum, and plasma. Both tests are inexpensive, rapid, and simple to perform. They are highly sensitive in the early stages of disease and their titre is used as an indirect measure of disease activity. They are widely used in screening programmes for detecting latent cases of the treponematoses. The RPR Teardrop Card Test is especially suitable for use in the field, since it can be performed using blood plasma collected in heparinized capillary tubes from a finger-prick.

Experience has shown that the prevalence of latent yaws, as measured by a nontreponemal antigen test in a given population, can be predicted by the prevalence of active yaws cases:

Percentage of population with active yaws	Percentage of seroreactors
1–2	8.5
11–15	54.0
16–20	71.0
23–30	77.5

Therefore, serological tests are seldom indicated in high prevalence areas except to confirm atypical cases of yaws.

The treponemal antigen tests are very sensitive and are reactive (the preferred term for a positive test) in the presence of only a few molecules of specific antibody. These tests are used to confirm a reactive nontreponemal antigen test when such confirmation is required.

Normal human serum may sometimes be reactive in both types of test. False-positive reactions of this sort can be avoided by diluting the serum in buffered saline before testing or, preferably, by first mixing the serum with a sorbent extracted from cultures of the avirulent Reiter strain of "*T. phagedenis*" (invalid); it is thought that this sorbent removes cross-reacting treponemal antibodies. The fluorescent treponemal antibody-absorption (FTA-ABS) test is a widely used treponemal antigen test.

A recent development in treponemal antigen tests is to use killed *T. pallidum* fixed to the surface of sheep or turkey erythrocytes to detect treponemal haemagglutinating antibodies in yaws or syphilitic serum (the *T. pallidum* haemagglutination assay (TPHA) and microhaemagglutination assay—*T. pallidum* (MHA-TP). As in the FTA-ABS test, cross-reactive treponemal antibody is removed with Reiter protein before testing. The haemagglutination tests have about the same sensitivity and specificity as the FTA-ABS test and may be preferred because they are more easily and rapidly performed than FTA-ABS and do not require expensive equipment.

False-positive reactions

The nontreponemal antigen tests sometimes give false-positive reactions with sera from patients who have no clinical evidence of syphilis, yaws, or pinta, and are nonreactive to treponemal antigen tests. Patients of this group are referred to as "false-positive" (FP) reactors. Acute FP reactions are those that become nonreactive in less than 6 months; they may be seen after smallpox vaccination, and in persons suffering from atypical pneumonia, malaria, or leprosy. Chronic FP reactions persist longer than 6 months, increase with age, and are common in patients with systemic lupus erythematosus or related connective-tissue disease. About 25 % of persons showing chronic FP reactions also have abnormal serum globulins, such as rheumatoid factor and antinuclear antibody. The FP reaction often begins before the onset of overt collagen-vascular disease, but the prognostic significance of this reaction in an otherwise healthy person is unknown.

Colour Plates

(Figures 3 – 70)

Fig. 3. Initial papillomatous yaws lesion on the upper thigh (also called primary framboesioma, mother yaw, *chancre pianique*). The initial lesion usually commences as a papule on the lower extremities and slowly enlarges to form a raspberry-like lesion.

Fig. 4. Papillomatous early yaws of the forearm with underlying osteoperiostitis. Inflammation of the periosteum makes the bones of the forearm very tender to palpation.

Fig. 5. Papillomata on the neck and hairline. Note the small satellite papules on the scalp.

Fig. 6. Initial papillomata of the axilla. Papillomata favour the humid areas of the body surface.

Fig. 7. Early yaws papillomata on the neck before treatment.

Fig. 8. Early yaws papillomata on the neck two weeks after treatment with 1.2×10^6 units of benzathine benzylpenicillin.

Fig. 9. Early yaws papillomata on the wrist—before treatment.

Fig. 10. Early yaws papillomata two weeks after treatment with
1.2×10^6 units of benzathine benzylpenicillin.

Fig. 11. Serpiginous papilloma of early yaws.

Fig. 12. Ulceropapillomatous early yaws with secondary papular lesions. The initial yaws lesion appeared on the buttock and was followed in two months by the appearance of papules.

Fig. 13. Ulceropapillomatous early yaws lesion on the chin with satellite papules. The central area of this lesion is covered by a thin, loosely adherent, yellow or grey, serous crust.

Fig. 14. Early ulceropapillomatous yaws on the leg (also called *ulcère post-chancreux*).
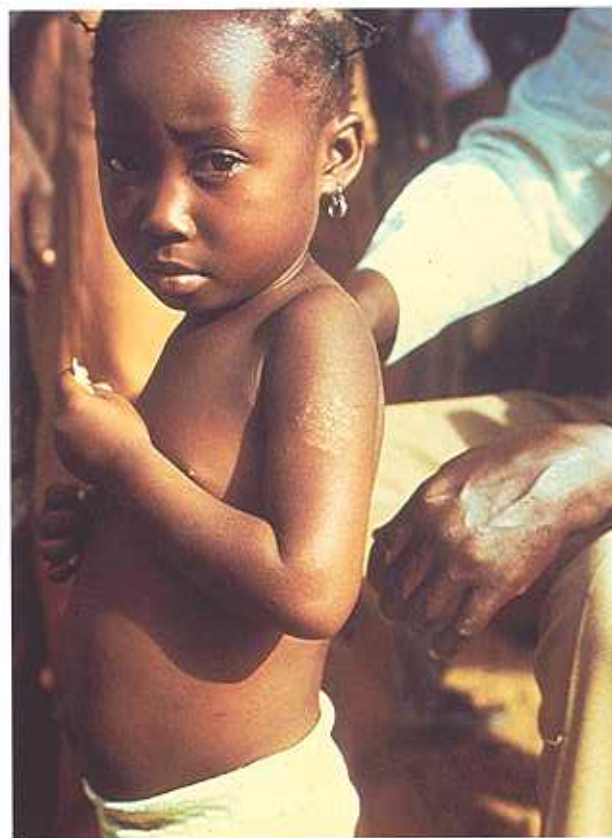
Fig. 15. Squamous macules of early yaws. Macules contain fewer treponemes and are not as infectious as papillomata.

Fig. 16. Squamous macules of early yaws.

Fig. 17. Squamous macular palmar yaws.

Fig. 18. Squamous macular plantar yaws.
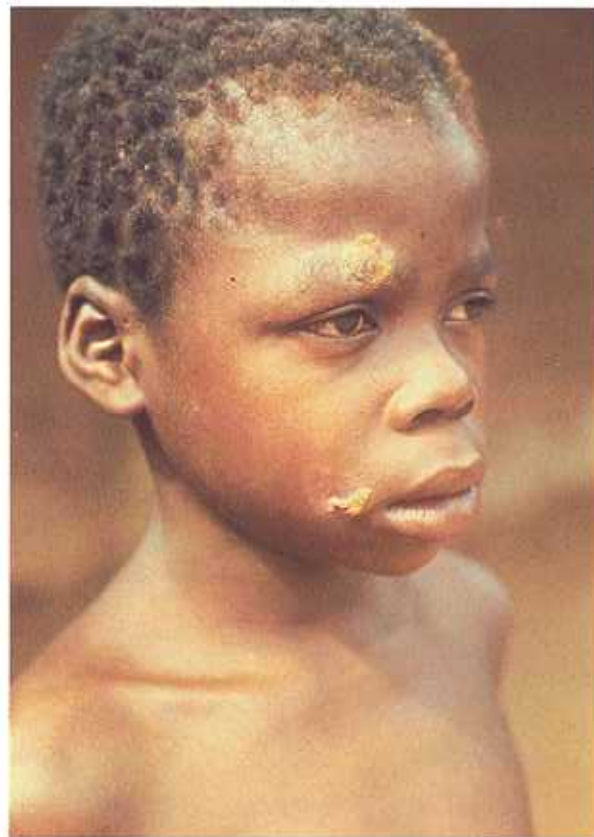
Fig. 19. Mixed squamous maculopapulomatous early yaws.

Fig. 20. Mucocutaneous early yaws. These lesions are identical to those found in early endemic syphilis. However, isolated oral mucosal lesions, while common in endemic syphilis, are very rare in yaws.

Fig. 21. Squamous micro-papules of early yaws with axillary papillomata. Left axilla and scapular area.

Fig. 22. Polymorphous early yaws.

Fig. 23. Nodular early yaws. These are most frequent on the front of the knees. They never develop into papillomata.

Fig. 24. Plaques of early yaws. These heal and leave no scar.

Fig. 25. Plantar papillomata with hyperkeratotic macular plantar early yaws ("crab" yaws). These lesions are painful.

Fig. 26. Hyperkeratotic macular plantar early yaws. A papilloma is present on the heel.
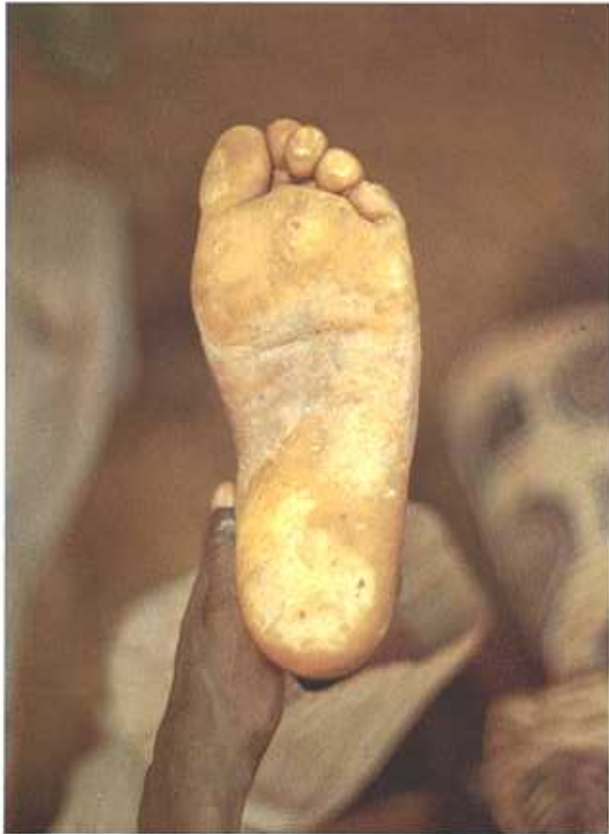
Fig. 27. Hyperkeratotic macular plantar early yaws.

Fig. 28. Hyperkeratotic macular palmar early yaws.
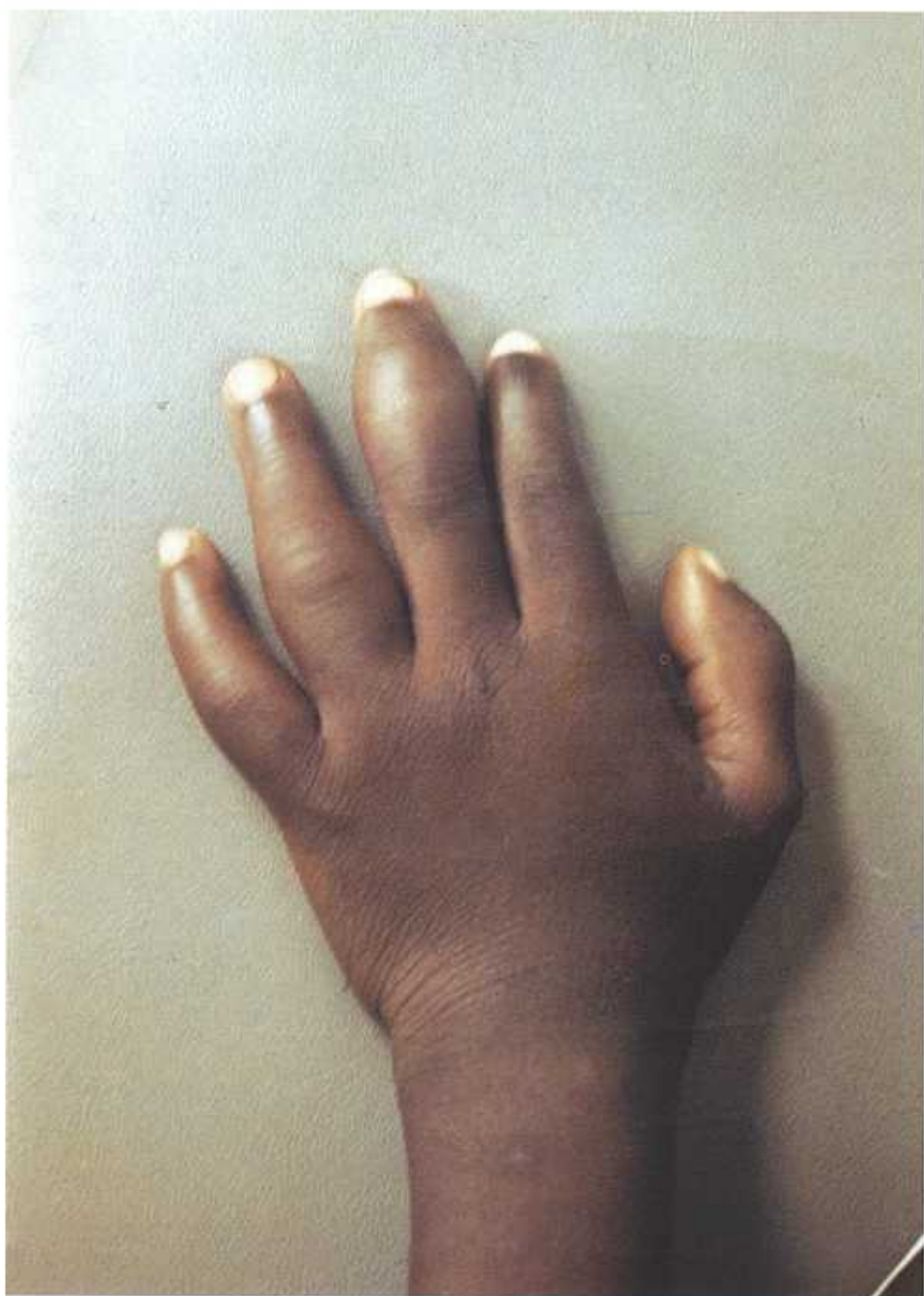
Fig. 29. Osteoperiostitis and polydactylitis of early yaws.

Fig. 30. Osteoperiostitis of early yaws of the tibia and fibula.

Fig. 31. Radiographic changes in early yaws osteoperiostitis (same patient as in Fig. 30).

Fig. 32. Scars from deep ulcerated nodular late yaws. Deformity is caused by osteoperiostitis and scar tissue.

Fig. 33. Gangosa (rhinopharyngitis mutilans). The lesion occurs
by direct extension of early mucocutaneous yaws lesions at the
borders of the nose and mouth into the nasopharyngeal mucous
membranes, followed by bacterial superinfection and ulceration.

Fig. 34. Gangosa. Although classified as a late yaws lesion, gangosa may occur 1–3 years after infection.

Fig. 35. Gangosa.

Fig. 36. Gummatous osteoperiostitis. Monodactylitis is characteristic of late yaws bone lesions. Deformity of the fingers may result.

Fig. 37. Sabre tibia. This irreversible condition is caused by chronic, untreated osteoperiostitis.

Fig. 38. Gondou. Gondou is a hypertrophic osteitis of the nasal process of the maxilla. It occurs in early yaws and may heal completely or leave a permanent scar.

Fig. 39. Juxta-articular nodules. These occur in late yaws and should not be confused with the nodules of early yaws.

Fig. 40. Impetigo. These lesions have an acute onset and may be filled with clear fluid which serves to distinguish them from secondary yaws.

Fig. 41. Impetigo.

Fig. 42. Tinea versicolor.

Fig. 43. Molluscum contagiosum.

Fig. 44. Scabies.

Fig. 45. Scabies.

Fig. 46. Lichen planus.
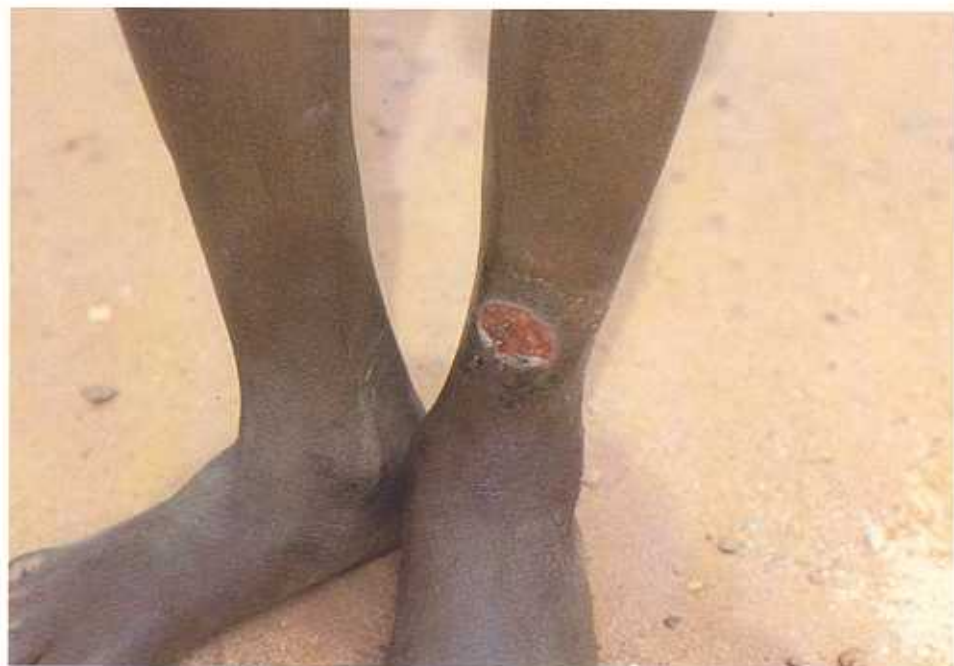
Fig. 47. Tropical ulcer.

Fig. 48. Plantar warts.

Fig. 49. Tungiasis.

Fig. 50. Cutaneous leishmaniasis.

Fig. 51. Leprosy.
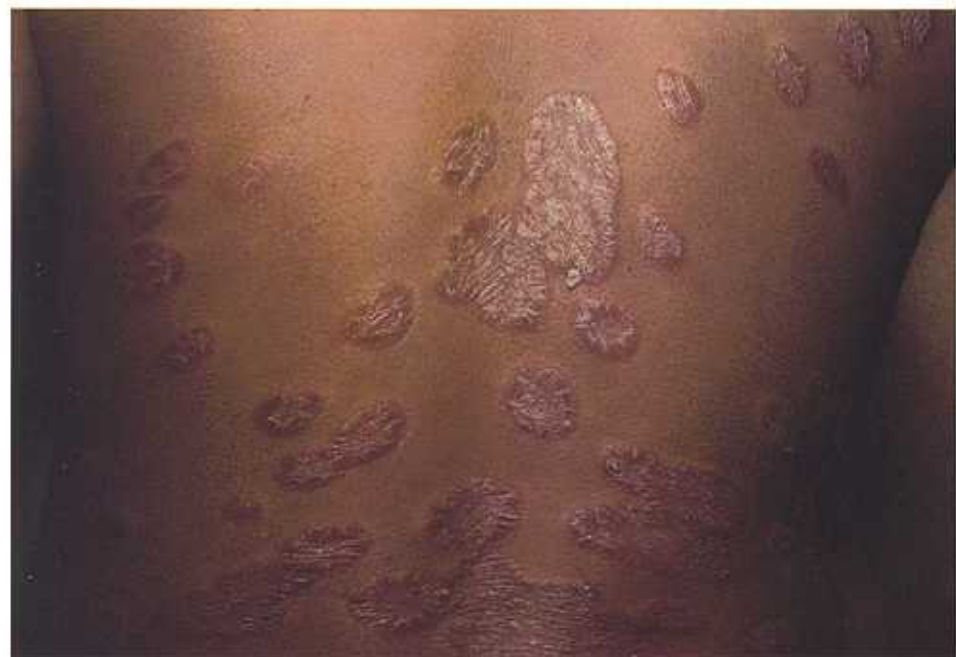
Fig. 52. Psoriasis.

Fig. 53. Psoriasis.

Fig. 54. Mucous patches of endemic syphilis. These are the most common initial lesions in endemic syphilis.

Fig. 55. Angular stomatitis (also called split papules) of endemic syphilis. These are also found in early yaws.

Fig. 56. Disseminated papules of secondary endemic syphilis.
This and other rashes of endemic syphilis cannot be
differentiated from those of venereal syphilis.

Fig. 57. Axillary condylomata of early endemic syphilis. Identical lesions are common in yaws.

Fig. 58. Late tertiary endemic syphilis—rhinopharyngitis mutilans.

Fig. 59. Chronic ulceration and depigmentation of late cutaneous endemic syphilis.

Fig. 60. Erythematosquamous plaque of early pinta. The initial pinta lesion is a papule which slowly enlarges to become a pruritic erythematous plaque resembling psoriasis. The legs and the dorsum of the foot are the most common sites of involvement.

Fig. 61. Violaceous psoriatic plaque of early pinta on the forearm. These lesions become pigmented with age, changing from copper to lead-grey to a slate-blue colour; after several years they become achromic.

Fig. 62. Late pigmented pinta, blue variety.

Fig. 63. Hyperpigmented, atrophic skin of late pinta.

Fig. 64. Achromic scars of late pinta. These scars may be confused with tinea versicolor or vitiligo when they occur on the trunk.

Fig. 65. Primary penile chancre of venereal syphilis.

Fig. 66. Papular rash of secondary venereal syphilis.

Fig. 67. Annular rash of secondary venereal syphilis on the face.

Fig. 68. Condylomata of the vulva and perineum in secondary venereal syphilis.

Fig. 69. *Treponema pallidum* seen under dark-field microscopy.

Fig. 70. Tissue biopsy showing *Treponema pallidum*.

Treatment of the treponematoses

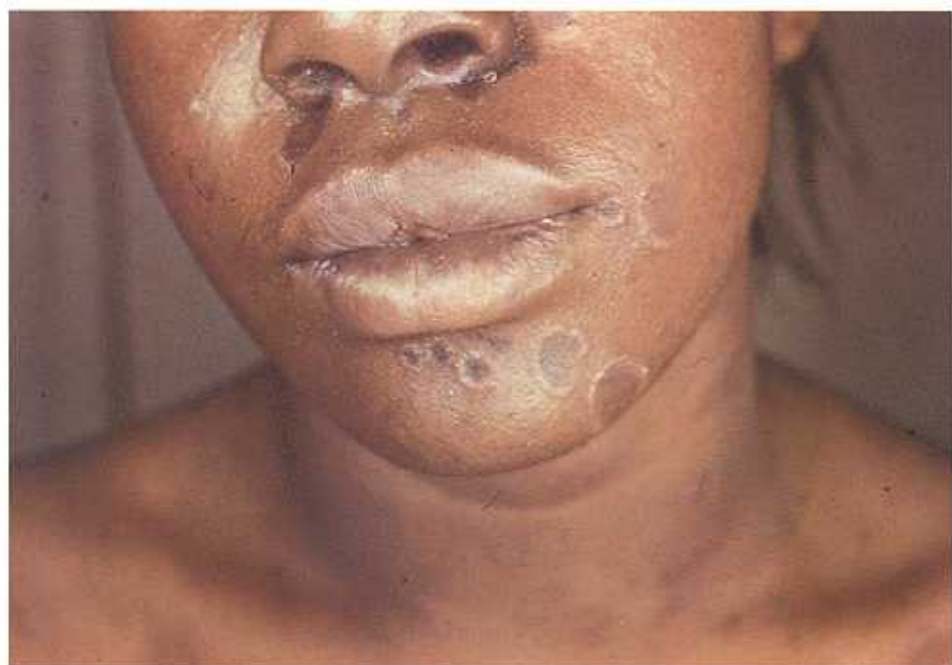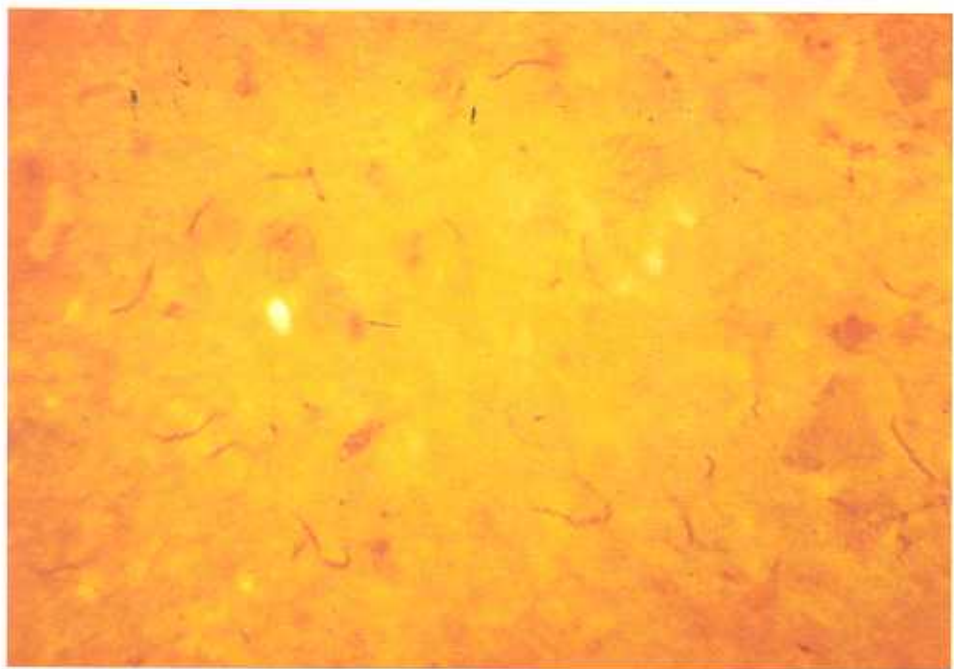The aim of treatment of the treponematoses is to halt the progression of disease by curing the infection. Tissue injury occurring during the early stages of infection resolves completely following adequate therapy, but tissue damage occurring during the late stages of infection is irreversible.

A number of drugs have been used to treat the treponematoses. The first drug to be manufactured for the treatment of venereal syphilis was arsphenamine, which was developed by Paul Ehrlich in 1909. Arsphenamine was also effective against yaws, but the drug was inherently toxic and a long series of weekly injections was needed to effect a cure. Penicillin proved to be highly effective against yaws and the other treponemal diseases in the 1940s, and it revolutionized the therapy of these infections. Tests on experimentally infected animals and infected patients showed that benzylpenicillin levels of 0.03 units per ml of serum or greater maintained for at least 7 days were treponemicidal. These levels can be achieved by giving repeated doses of short-acting benzylpenicillin preparations such as aqueous benzylpenicillin, or by a single intramuscular injection of the slowly absorbed, repository benzylpenicillin preparations such as benzathine benzylpenicillin or penicillin aluminium monostearate (PAM).

A recent WHO Expert Committee on Venereal Diseases and Treponematoses has recommended benzathine benzylpenicillin in preference to the other forms of penicillin for the treatment of treponemal diseases.[1] PAM was the mainstay of the yaws campaigns of the 1950s and 1960s, but this preparation is no longer being manufactured in sufficient quantity for present-day use. Moreover, the serum concentration of penicillin produced by benzathine benzylpenicillin persists above the treponemicidal level much longer than that produced by PAM. A single intramuscular injection of $2\cdot4 \times 10^6$ units of benzathine benzylpenicillin in a healthy, ambulatory adult produces a penicillinaemia above the treponemicidal level for more than 3 weeks, sufficient not

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[1] WHO Technical Report Series (Sixth report of the WHO Expert Committee on Venereal Diseases and Treponematoses) (in press).

only for curing treponemal diseases but also for providing protection against reinfection during this period.

The recommended schedules for treatment of the endemic treponematoses (not including venereal syphilis) are 600 000 units of benzathine benzylpenicillin for all cases and contacts aged under 10 years, and 1 200 000 units for those aged over 10 years. Benzathine benzylpenicillin is given as a single intramuscular injection, usually in the upper quadrant of the buttock. This drug is available in vials containing 12×10^6 units, and can be reconstituted to a concentration of 400 000 units per ml. Oral penicillin preparations should not be used, since intestinal absorption of the drug is highly variable and it is difficult to ensure that the drug will be taken as prescribed.

Large doses of benzathine benzylpenicillin are required to cure primary and secondary syphilis and even larger doses and prolonged treatment with shorter-acting penicillins are needed to cure the late visceral, neurological, and cardiovascular stages of the disease, because by that time the treponemes have invaded tissues that are difficult for penicillin to penetrate. Recommended treatment schedules for venereal syphilis are listed in Annex 4.

Once injected, penicillin begins to kill treponemes within minutes and the lesions are rendered noninfectious within 18–24 hours. However, an estimated 1–3 % of patients with yaws and endemic syphilis are probably not cured by the recommended treatment doses of penicillin. Reasons for treatment failure include: (a) use of penicillin preparations that do not conform to WHO standards for purity and activity; and (b) use of penicillin preparations whose level of activity has been lowered by improper storage, reconstitution, or use beyond the expiration date. Finally, the possibility of reinfection should always be kept in mind—in hyperendemic areas, the probability of reinfection is inversely related to the level of population coverage achieved during the initial mass treatment.

The success of mass treatment against yaws depends on the level of population coverage. Since yaws is transmitted by direct, person-to-person non-sexual contact (see Chapter 2), all contacts of infectious cases must be treated if yaws is to be eliminated from the community. It should be borne in mind that many contacts of yaws patients become infected with the disease, and while symptomless, have either incubating or latent infection. In either case, many of the contacts will develop or relapse with infectious yaws lesions, thereby exposing recently treated patients to reinfection.

Theoretically, treatment failure could also be caused by infection with strains of pathogenic treponemes that are relatively resistant to penicillin. Although this phenomenon has occurred with many other bacteria such as the gonococcus and pneumococcus, there are no documented instances of penicillin-resistant treponemes. However, there is no practical mechanism for the routine testing of the penicillin susceptibility of pathogenic treponemes.

Penicillin treatment always carries the risk of serious side-effects, including fatal anaphylaxis. During the initial mass treatment campaigns

with penicillin, almost all of those treated were receiving penicillin for the first time and were at low risk of developing serious side-effects such as anaphylaxis, serum sickness, or haemolytic anaemia. Those giving mass treatment should be prepared to treat such drug reactions with epinephrine, antihistamines, or other drugs, depending upon the nature of the individual reaction.

Very little information is available on the treatment of yaws, endemic syphilis, and pinta with other antibiotics in patients allergic to penicillin. Tetracycline or erythromycin, 500 mg by mouth four times daily for 15 days, is probably an effective treatment. Children between 8 and 15 years of age may be given half doses of either drug, and those under 8 years of age should be given only erythromycin in doses adjusted for their body weight. *Tetracycline is not recommended for pregnant women because it may cause hepatic and skeletal problems in the mother and fetus, respectively.*[1]

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[1] WHO Technical Report Series, No. 674, 1982.

Control of the endemic treponematoses[1]

The experience gained from national yaws control programmes, and WHO-sponsored pilot yaws control projects between 1946 and 1952 resulted in the following set of principles and procedures that should be followed to ensure the control or eradication of yaws in any given community:

— All stages of the control programme, including consolidation and maintenance activities, should be planned and financed from the outset.
— The whole available population should be seen and evaluated at the initial treatment survey.
— Treatment with long-acting penicillin should be given to all active cases when they are diagnosed and to all latent cases and contacts of the patient in accordance with the three treatment policies stated below (Table 5).
— Periodic surveys to detect and treat new or missed cases, relapsed cases, and treatment failures are essential.
— The control activities should be expanded in such a way that an ever-enlarging compact area of control is formed, so that reintroduction of yaws by patients coming from untreated areas is minimized.

Mass treatment is simple in theory but difficult and expensive in practice. Logistics are often the limiting factor. Hence, careful attention must be given to the planning aspect of the control activities from their inception to the end of the maintenance phase.

Planning

The first step is to document the prevalence and extent of yaws in a given geographical area in order to obtain the data necessary for planning the control activities. Clinical surveys for active yaws (or endemic syphilis or pinta) may be conducted without any sophisticated laboratory test. A portable dark-field microscope and/or the RPR Teardrop Card Test for

[1] Although this chapter deals mainly with the control of yaws, the basic principles discussed here also apply to the control of endemic syphilis and pinta.

measuring treponemal antibody can provide useful diagnostic information on the spot during village surveys.

The number of active clinical cases of yaws in a given community usually increases 2-3 fold in the wet season, probably because more infections relapse at this time. Therefore, in areas with distinct wet and dry seasons, the prevalence of active yaws found in surveys conducted in the dry season should be at least doubled before the treatment policy is established.

Surveys for latent disease require serological tests. Measuring yaws antibody in the entire population is impracticable, but serological surveys of a sample of the population, can provide accurate information about the prevalence of latent disease. A method for choosing a sample population is given in Annex 5.

Treatment policies

The extent of treatment given to a community, village, or other group living close to one another is based on the prevalence of clinically active yaws in the community (Table 5). Only simultaneous treatment of active, incubating and latent infections will destroy the reservoir of disease. *An evaluation of the total prevalence of yaws in the entire population is essential to determine the correct treatment policy.* In isolated and remote villages, total mass treatment may be appropriate even if the prevalence of active yaws is less than 10% (penicillin is the least expensive component of mass treatment at the village level).

Table 5. WHO treatment policies for yaws

Approximate prevalence of clinically active yaws in the community	Recommended treatment
High—over 10% (hyperendemic)	Give benzathine benzylpenicillin to the entire population (total mass treatment; TMT)
Medium—5–10% (mesoendemic)	Treat all active cases, all children under 15, and obvious contacts of infectious cases with benzathine benzylpenicillin (juvenile mass treatment; JMT)
Low—under 5% (hypoendemic)	Give benzathine benzylpenicillin to all active cases and all household and other obvious contacts (selective mass treatment; SMT)

Contacts are defined as those people who have frequent, direct, person-to-person contact with a patient with active yaws lesions. These people are assumed either to be incubating yaws or to have latent disease. As such they may develop or relapse with infectious yaws lesions 2-3 times or more during the first 5 years of disease and serve as a

source of new infections. Although latent cases can only be diagnosed by serological testing, this is seldom practicable or necessary. Most latent and incubating cases are found in clusters centred around an infectious case, and this allows penicillin treatment to be targeted.

Planned yaws treatment should be advertised to the general public. It is critically important to notify in advance the population in an area where treatment is to be given. This is best done by sending an official to the selected villages ahead of the rest of the team. Other tasks of the official include: (*a*) obtaining the cooperation of the village leaders; (*b*) soliciting the assistance of local village health workers; and (*c*) taking a census of the village population. The date the control team is to visit the village should be established well in advance in order to ensure that as few residents as possible will be away from the village on that day.

Surveillance

Surveillance is the key element of yaws control. Periodic resurveys are essential and should begin 6 months after the initial treatment survey. The timing of the subsequent resurveys should depend upon the prevalence—in areas of high prevalence they should be more frequent than in areas of low prevalence—but will usually need to be done at least every 2 years. During surveillance resurveys in several campaigns, very few active yaws cases were found among persons who had received treatment at the initial treatment survey; however, more were found among persons who were away from the village at the time of the initial survey or who had moved into the village since then. Thus, even if the chemotherapeutic agent used produced complete clinical and serological cure in all patients treated, resurveys would still be needed to find and treat active cases (which will be most numerous in persons not previously treated) and to protect the immediate contacts of the infectious cases.

Maintenance phase

So long as cases of endemic treponematosis exist in a country, control measures of some sort will be necessary to prevent a resurgence of infection. Following mass treatment or selective epidemiological control efforts, anti-treponematosis activities can never safely be relaxed entirely unless the infection has been eliminated. In these and other situations where an endemic treponematosis is known to be present in a country, but neither mass treatment nor selective epidemiological control seems indicated or can be undertaken, a coordinated maintenance level of activity is required. Planning for maintenance level activities to follow mass treatment or selective epidemiological control efforts should begin at the same time as the aggressive control actions.

Every effort should be made to incorporate the maintenance phase of mass treatment into the primary health care infrastructure serving the population infected with yaws (or other treponematosis). The training and resources needed to enable the primary health care workers to conduct surveillance, treat active cases, and contain focal epidemics must be provided.

Selective epidemiological control

In some situations, where the prevalence of yaws or other endemic treponematoses is not great enough to require a full-scale treatment effort, it may still be appropriate to undertake an aggressive, but discriminating approach in order to reduce the disease to a level where it no longer constitutes a public health problem. Thus, a country could enhance its surveillance system for yaws (or another treponematosis) by using health workers and others to seek actively for suspected cases of endemic treponematosis, rather than relying on passive reporting. At the same time, all reported or suspected cases of endemic treponematosis should be rapidly evaluated, and if the diagnosis is confirmed, the case and contacts should be treated. All cases should be investigated in order to determine the source and spread of infection. Such an approach would be similar to the selective mass treatment policy recommended for areas of low prevalence (<5%) of active yaws; the only difference is that active surveillance and rapid response on a national and local level are important features of selective epidemiological control. This type of approach to control has not been applied on a large scale in the case of endemic treponematoses, but was used effectively to control smallpox.

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A glossary of medical terms used in this handbook

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achromia: absence of normal skin colour

anaesthesia: loss of feeling or sensation

annular: ring-shaped

antibody: any protein produced in the body in response to the presence of an antigen, with which it combines

antigen: a substance that can cause the formation of specific antibodies and that can react specifically with antibodies

atrophy: a wasting away of a body part or tissue

autoinoculation: spread of infection from one part to other parts of the same body

avirulent: not virulent

bulla: an elevated, fluid-containing lesion more than 5 mm in diameter, e.g., pemphigus; it is larger than a vesicle;

circinate: circular

circumoral: around or near the mouth

confluent: becoming merged; not discrete

constitutional symptoms: symptoms indicating a disorder of the whole body

cutaneous: pertaining to the skin

discoid: shaped like a disc

discrete: separated, or characterized by, lesions that do not merge

disseminated: scattered or distributed over a large area

dyschromia: any disorder of skin pigmentation

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endemic: (of disease) constantly present in a given community

eradication: the process of completely eliminating or getting rid of a disease

erosion: a wearing away of a tissue; a kind of destructive ulceration

erythrocyte: red blood cell

exudate: material (e.g., fluid) discharged from lesion

fissure: a narrow opening or crack in the skin

flora: the bacterial content

focus (foci): centre of activity or disease

fomes (or fomite; pl. fomites): any object capable of transmitting an infectious agent

framboesioma: the primary lesion of yaws consisting of a large single projecting papule

generalized: affecting many or all parts of the body; not local

genital: of or relating to the sexual organs

globulin: a class of serum proteins containing antibodies

gumma (pl. gummata): a soft growth or tumour having a rubbery consistency

haemagglutination: adhesion of red blood cells to each other

hyper-: prefix meaning above normal or excessive

hypo-: prefix meaning below normal

induration: the process of hardening of a tissue or organ

intertriginous: affected with superficial dermatitis occurring on opposed surfaces (e.g., anal clefts)

in vitro: in a test-tube or other container

keratosis: an area of skin marked by overgrowth of horny tissue

leukoderma: patches of abnormal whiteness on the skin

lesion: a sore or injury caused by disease or infection

linear: pertaining to or resembling a line

macule: a skin lesion without elevation or depression that can be seen but not felt; usually discoloured by pigmentary changes, e.g., measles, freckles

maculopapules: lesions with both macular and papular elements; these appear as slightly elevated areas or flat areas containing papules

malaise: an indefinite feeling of debility or discomfort accompanying the onset of illness

meso-: a prefix signifying *middle* or intermediate

metastasis: the transfer of an infection or disease from its primary site to other parts of the body

morphology: the form and structure of an organism and its parts

mucosa: a mucous membrane

multiform: occurring in several forms; polymorphous

nocturnal: occurring at night

nodule (nodular): a solid, elevated lesion over 5 mm in diameter, e.g., lichen planus, lesions of secondary syphilis

opportunistic pathogen: an organism that is not normally pathogenic but can cause disease if resistance is lowered e.g., the avirulent treponemes that sometimes become pathogenic in persons infected by the virulent *T. pallidum*

oropharynx: the part of the pharynx between the soft palate and the upper edge of the epiglottis

osteitis: inflammation or infection of bones

overt: open to view; manifest

palmar: of or on the palm

papilloma: dry or moist lesion composed of hypertrophic papillae (small, nipple-shaped projections), 5–25 mm in diameter, e.g., yaws lesion, wart

papule: any solid, small elevation of the skin 2–5 mm in diameter, e.g., acne

pathogen: any infectious agent capable of causing disease

peri-: a prefix meaning around or about (perianal, periaxillary)

periosteum: the membrane that closely covers all bones except at articular surfaces

plantar: of or pertaining to the sole of the foot

plaque: raised areas of skin formed by merging of a number of papules, e.g., psoriasis

polymorphous: having many forms

purulent: containing or consisting of pus

pustule: a solid, elevated lesion, up to 5 mm in diameter, containing pus, e.g., boils, pyoderma

recrudescence: the reappearance of symptoms of a disease after a period of inactivity

reservoir: any natural source of repeated infection, such as an asymptomatic infected person

serous: resembling or pertaining to serum

serpiginous: snake-like or creeping, having a wavy or much indented margin

sporadic: occurring occasionally, singly, or in scattered instances

squamous: covered with or consisting of scales

stomatitis: inflammation of the oral mucosa; *angular stomatitis* involves superficial erosions and fissuring at the angles of the mouth

sulcus: a groove, trench, or furrow

suppuration: the act of forming and discharging pus

surveillance: close watch kept over a disease

titre: (in serology) a measure of the amount of a specific antibody present in a given serum

treponemicidal: capable of killing treponemes

ulcer: a disintegration the surface of the skin or of a mucous membrane resulting in an open sore exposing deeper tissue

venereal: of or related to sexual intercourse

vesicle: an elevated, fluid-containing lesion or blister up to 5 mm in diameter, e.g., blisters caused by poison ivy

viable: capable of living

virulence: the degree of pathogenicity of an infectious agent as indicated by the number of people who develop serious complications or die following infection

viscus (pl. viscera): a term for the internal organs (such as the heart, liver, or intestine) located in any one of the three great cavities of the body

Dark-field microscope technique

To permit the viewing of treponemes, an ordinary compound microscope has to be equipped with a dark-field condenser. This condenser has an opaque stop which blocks out the direct light rays, allowing only the peripheral rays to pass through. These are then directed on to the specimen at an acute angle (see Annex 2, Fig. 1). The objective lens must have a funnel stop to reduce its aperture. Light rays from the condenser do not reach the eye of the examiner until an object, such as a red blood cell or a treponeme, deflects the light rays directly into the objective and through the barrel of the microscope to the eyepiece. With this system the pathogenic treponemes appear under the microscope as thin, tightly-coiled, silver threads on a black background (Fig. 69).

Specimen collection

All suspicious clinical lesions should be regarded as infectious and treated accordingly. The lesion should be cleaned thoroughly with gauze swabs soaked in saline, protective gloves being worn. This usually provokes an adequate flow of serous discharge from the lesion. Dry or healing lesions may have to be scraped with a scalpel and squeezed to make the serum flow. A microscope coverslip held between the gloved thumb and index finger or with a pair of forceps is touched to the surface of the lesion so that a drop of serum adheres to it. The coverslip with the drop of serum on its undersurface is placed firmly on a thin glass slide. Alternatively, the specimen may be aspirated from the lesion, or an enlarged lymph node, with a sterile needle and syringe; the serum is then expelled directly on to the slide and covered with a coverslip. The specimen should be examined under the microscope as soon as possible.

Method of examination

Place the slide containing the specimen on the mechanical stage, the coverslip being on top. With the dark-field condenser in position and the

substage iris diaphragm open, adjust the mirror to reflect the maximum amount of light into the objective lens. Put one drop of immersion oil on the condenser lens and then raise the condenser lens until it just touches the lower surface of the microscope slide. Low-power objectives (10 ×, 40 ×) should be used to bring the field into focus. Lighting will be scant but cellular debris can be identified. By raising and lowering the dark-field condenser very gently, bring a uniform circle of light into view. This light should be centred in the field of vision by using the adjustment screws on the sides of the substage condenser. Place a drop of immersion oil on the coverslip and lower the oil-immersion objective into the oil. Finally, fine focusing and minor adjustments in the height of the substage condenser and in the position of the slide may be necessary to achieve a uniformly dark field in which brilliant objects can be seen.

Before attempting the examination of clinical specimens, skilled use of the technique can be learned by practising frequently using preparations obtained from the gum margins of healthy people; these specimens contain avirulent "*Treponema microdentium*" (invalid) and "*T. macrodentium*" (invalid). These two treponemes can be identified by observing their spirals, which in the case of the former are shallower, shorter, and more angular than *T. pallidum* or *T. pertenue*, and in that of the latter are fewer, more irregular, and larger than those of the pathogenic treponemes.

Annex 2, Fig. 1. The technique of dark-field microscopy
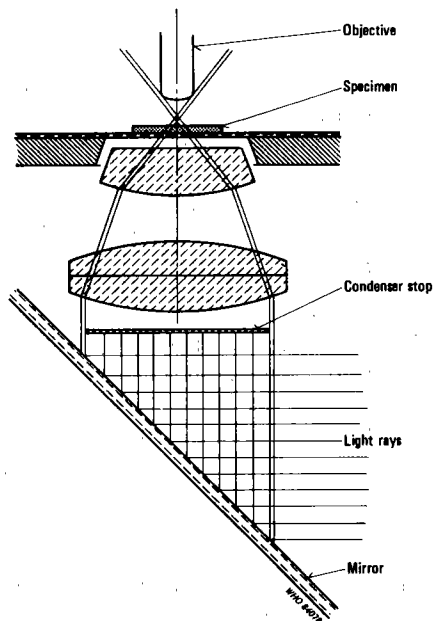
Laboratory procedures for the RPR card and VDRL tests[1]

Specimen collection

The collection tubes for venous blood should be clean, dry, and sterile in order to prevent contamination and haemolysis of the specimen.[2] Vacuum tubes or tubes with paraffin-coated corks may be used.

Place a tourniquet around the upper arm to block the return of venous blood from the forearm and hand. Clean the skin overlying one of the antecubital veins with an alcohol swab and draw 5–8 ml of blood. Place the blood in the sterile tube, and allow to clot at ambient temperature. Centrifuge the specimen at 450 g (approximately 2000 r/min for a centrifuge of 10-cm radius) for 10 minutes and remove the serum. (Alternatively, allow the blood clot to retract over a period of 6–8 hours, and decant the serum.) The serum should be stored in a separate tube at −4°C until it can be sent to the laboratory. Specimens should not be sent to the laboratory over long weekends or holidays.

Be sure to label the specimen with the patient's name or identifying number and the date of collection. Ensure that the label is firmly attached and will not be dislodged during handling. Also indicate whether or not the specimen was heated (giving time and temperature) to inactivate complement.

Preparation and use of control sera

Control sera of graded reactivity should be included each time a serological test is performed. For the nontreponemal antigen flocculation tests with serum (i.e., VDRL, RPR) the antigen suspension to be used each day is first examined against the control sera. The results obtained with the controls should conform to the established reactivity pattern. If

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[1] Adapted from: *Manual of tests for syphilis 1969.* Washington, DC, US Government Printing Office, 1969. This manual is available on request from: Centers for Disease Control, Atlanta, GA 30333, USA.

[2] Haemolysis may be caused by: wet or dirty syringes, needles, or tubes; chemicals; freezing; or extreme heat.

the results are not acceptable, routine testing should be delayed until the correct reactivity has been established (by preparing another antigen suspension, correcting room temperature, adjusting equipment, etc.). The control sera should also be included in the treponemal antigen FTA-ABS and haemagglutination tests. If the pattern of reactivity is not acceptable, the results of the test for the individual specimens should be considered invalid and should not be reported.

Control sera of graded reactivity for nontreponemal and treponemal antigen test procedures are available from commercial sources or may be prepared from individual serum samples or from serum samples pooled after testing. A pattern of reactivity should be established for each new lot of control serum prepared in the laboratory, or confirmed for each new lot of control serum obtained from a commercial source, by comparing the new control serum (or sera) with a standard control serum.

Use of safety pipetting devices for syphilis serology

In keeping with the recommendation to eliminate pipetting by mouth of possibly infectious serum samples, studies to determine the efficacy of substitutes for pipettes have been made by the Centers for Disease Control, Atlanta, GA, USA. The following are some recommendations for measuring sera for qualitative and quantitative tests.

(1) Safety pipetting devices that deliver approximately 0.05 ml of serum per drop when held in a vertical position may be used for the RPR card and VDRL qualitative tests.

(2) Safety pipetting devices with disposable plastic tips that deliver 0.05 ml may be used for preparing serial twofold dilutions of serum for the RPR (18-mm circle) card and VDRL quantitative tests.

(3) Each laboratory should determine the type of safety pipetting device it prefers on the basis of accuracy of measurement, reproducibility, ease of use, and cost.

Rapid plasma reagin (RPR) (18-mm circle) card test of serum

Equipment, glassware and reagents

With the exception of the control sera, rotating machine, and humidifier cover, all equipment and supplies necessary for performing the RPR (18-mm circle) card test are contained in a kit. The test kit contains:

— RPR card test antigen. This antigen contains a suspension of specially prepared charcoal particles. Store the antigen suspension in ampoules or in the plastic dispensing bottle at 2–8 °C. An unopened ampoule has a shelf-life of at least 12 months from the date of manufacture; the antigen suspension stored in the plastic dispensing bottle and kept refrigerated usually remains satisfactory for ap-

proximately 3 months. Do not use the antigen suspension beyond the expiry date shown on the ampoule. A new lot of antigen suspension should be compared with an antigen suspension of known reactivity before being placed in routine use.
— 20-Gauge (0.9 mm) needle without bevel.
— Plastic dispensing bottle.
— Plastic-coated cards, each with ten 18-mm circular spots.
— Safety pipetting devices that deliver 0.05 ml per drop, or capillary pipettes of 0.05 ml capacity.
— Rubber bulbs.
— Stirrers.

Additional equipment includes a rotating machine of fixed-speed (or of adjustable speed up to 100 r/min) capable of circumscribing a circle of 1.9 cm diameter on a horizontal plane. Any convenient humidifier cover containing a moistened blotter may be used to cover the cards during rotation.

Testing the accuracy of delivery needles

It is of primary importance to use the proper amount of reagents. For this reason, the needles used should be checked every day.

For the RPR (18-mm circle) card test, antigen suspension should be dispensed from a plastic dispensing bottle with a 20-gauge (0.9 mm) disposable needle without bevel. These needles should deliver 60 ± 2 drops of antigen suspension per ml when held in a vertical position. Practice will allow rapid delivery of antigen suspension, but care should be exercised to ensure that the drops are of uniform size.

To check the accuracy of the needle, place it on a 2-ml syringe or a 1-ml pipette. Fill the syringe or pipette with the antigen suspension and, holding it in a vertical position, count the number of drops delivered from 0.5 ml of antigen suspension. The needle is considered to be satisfactory if 30 ± 1 drops are obtained from 0.5 ml of suspension. A needle not meeting this specification should be replaced.

Preliminary testing of antigen suspension

Attach the needle hub to the tapered fitting on the plastic dispensing bottle. Shake the antigen ampoule to resuspend antigen particles, snap the ampoule neck at the break-line, and, by collapsing the bottle and using it as a bulb, withdraw all the antigen suspension into the dispensing bottle by suction. Shake the dispenser gently before each series of antigen drops is delivered.

Perform an RPR (18-mm circle) card test on the control sera of graded reactivity every day, as described below. Use only those suspensions that give the designated reactions with the control samples.

Preparation of sera

Centrifuge blood specimens at room temperature and at a force sufficient to separate the serum from the cells. Generally, 420–450 *g* for 5 minutes is satisfactory. The sera should be tested without heating and should be at 23–29 °C at the time of testing. Specimens may be retained in the original collection tube.

Test procedure

Slide flocculation tests for syphilis are affected by room temperature. For reliable and reproducible results, the control sera, RPR card antigen suspension, and test specimens should be at room temperature 23–29 °C when the tests are performed.

(1) Place 0.05 ml of unheated serum on to an 18-mm circle of the test card, using a safety pipetting device or a 0.05-ml capillary pipette with attached rubber bulb.

(2) Spread the serum sample with the inverted safety pipetting device (closed end) or a stirrer (broad end) to fill the entire circle.

(3) Add 1 drop (1/60 ml) of RPR card test antigen suspension to each test area containing serum. Do not stir.

(4) Place the card on the rotator and cover with the humidifier cover.

(5) Rotate the card for 8 minutes at 100 r/min on a mechanical rotating machine.

(6) Read the tests without magnification immediately after rotation. A brief rotating and tilting of the card by hand should be used to aid in differentiating nonreactive sera from weakly reactive sera.

(7) Report results as follows:

Reading	Report
Small to large clumps	Reactive (R)
No clumping or very slight roughness	Nonreactive (N)

Specimens giving any degree of clumping should be subjected to further serological study, including quantification.

(8) Upon completion of the daily tests, remove the needle, rinse it in water, and air-dry it. (Avoid wiping the needle, as this may remove its silicone coating). Recap the dispensing bottle and store it in a refrigerator.

Rapid plasma reagin (RPR) teardrop card test of serum

Equipment and reagents

All equipment and reagents needed to perform the test are included in the test kit. Instructions on how to perform the test are also provided with the kit. The only difference between this test and the 18-mm circle test is that a paper card containing teardrop-shaped areas for mixing test serum and antigen is used here in place of the plastic-coated card with

18-mm circular spots. The test is qualitative and can be performed on unheated serum or plasma samples. It is suitable for field use.

Test procedure

(1) Place a 0.05-ml drop of unheated test serum on to an open teardrop-shaped area on the card using the capillary pipette with attached rubber bulb provided with the kit.

(2) Add exactly 1 drop (1/60 ml) of RPR card test antigen suspension to each test serum.

(3) Stir the antigen-serum mixture with a toothpick (which must then be safely destroyed) and spread it to the margins of the teardrop.

(4) Rotate the teardrop card slowly by tilting the card by hand for 3 minutes.

(5) Read the test results immediately by visual examination. Compare the degree of clumping of the test serum with RPR antigen with the reactive, weakly reactive, and nonreactive examples illustrated in the instructions, or run known reactive sera in parallel with the test serum.

Venereal disease research laboratory (VDRL) slide tests

Equipment

— A rotating machine adjustable to 180 r/min, circumscribing a circle 1.9 cm in diameter on a horizontal plane.

— A ringmaker, to make paraffin rings of approximately 14 mm in diameter.

— A slide holder, for 5 cm × 7.5 cm microscope slides.

— Hypodermic needles, without bevels, 18-gauge (1.2 mm).

Glassware

— Slides, 5 cm × 7.5 cm, with 12 paraffin rings[1] approximately 14 mm in diameter, for serum test.

— Syringe, Luer-type, 1.0- or 2.0-ml.

— Bottles, 30-ml, round, glass-stoppered, narrow-mouth, approximately 35 mm in diameter, with flat inner bottom surfaces.

Note that some of the bottles now available are unsatisfactory for preparing antigen suspension because the convex inner bottom surface causes the saline to be distributed only at the periphery.

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[1] Glass slides with ceramic rings may also be used, provided that the rings are high enough to prevent spillage when the slides are rotated at the prescribed speeds. The slides must be clean so that the serum will spread to the inner surface of the ceramic rings. This type of slide should be discarded if the ceramic rings begin to flake off.

Reagents

(1) VDRL antigen.

(a) The antigen for this test is a colourless, alcoholic solution containing 0.3 ml/l cardiolipin, 9 ml/l cholesterol, and sufficient purified lecithin to produce standard reactivity. (In recent years, the amount of lecithin has been 2.1 ±0.1 ml/l.) Each lot of antigen must be serologically standardized by proper comparison with an antigen of known reactivity.

(b) The antigen is dispensed in screw-capped bottles or hermetically sealed glass ampoules, and should be stored in the dark either in a refrigerator (6–10 °C) or at room temperature. The components of this antigen remain in solution at these temperatures, so that any precipitate noted will indicate changes resulting from factors such as evaporation or contamination from pipettes. Antigen containing a precipitate should be discarded.

(c) A new lot of antigen should be compared with a standard antigen before being accepted for routine use. Testing should be performed on more than one day with control sera, individual sera of graded reactivity, and nonreactive sera. Reportable test results on individual specimens in qualitative and quantitative tests should be comparable with results obtained with the standard reagent.

(2) VDRL buffered saline containing 10 g/l sodium chloride, pH 6.0 ±0.1.

Formaldehyde, neutral (reagent grade)	0.5 ml
Disodium hydrogen phosphate (Na_2HPO_4), anhydrous (reagent grade)	0.037 g
Potassium dihydrogen phosphate (KH_2PO_4) (reagent grade)	0.170 g
Sodium chloride (reagent grade)	10.0 g
Distilled water	1000.0 ml

Check the pH of the solution and store it in screw-capped or glass-stoppered bottles.

Note: When an unexplained change in test reactivity occurs, check the pH of the buffered saline to determine if this is a contributing factor. Saline outside the range of pH 6.0 ±0.1 should be discarded.

(3) Saline containing 9 g of sodium chloride per litre. Add 900 mg of dry sodium chloride (reagent grade) to each 100 ml of distilled water.

(4) Saline containing 100 g of sodium chloride per litre. Add 10 g of dry sodium chloride (reagent grade) to each 100 ml of distilled water.

Preparation of antigen suspension

The temperature of the buffered saline and antigen should be in the range of 23–29 °C when the antigen suspension is prepared.

(1) Pipette 0.4 ml of buffered saline into a 30-ml round glass-stoppered bottle.

(2) Add 0.5 ml of antigen (from the lower half of a 1.0-ml pipette graduated to the tip) directly to the saline while continuously but gently rotating the bottle on a flat surface.

Note: The antigen is added drop by drop, but rapidly, so that approximately 6 seconds are allowed for every 0.5 ml of antigen. The tip of the pipette should remain in the upper third of bottle, and the rotation should not be vigorous and should not cause the saline to splash onto the pipette. The proper speed of rotation is obtained when the centre of the bottle circumscribes a 5-cm diameter circle approximately 3 times per second.

(3) Blow the last drop of antigen from the pipette without touching the saline with pipette.

(4) Continue the rotation of the bottle for 10 seconds.

(5) Add 4.1 ml of buffered saline from a 5-ml pipette.

(6) Replace the cap on the bottle and shake from bottom to top approximately 30 times in 10 seconds.

(7) The antigen suspension is ready for use and may be used during one day.

(8) A double volume of the antigen suspension may be prepared at one time by using doubled quantities of antigen and saline. A 10-ml pipette should be used to deliver the 8.2 ml of saline required. If larger quantities are required, more than one lot of antigen suspension should be prepared. Test each batch of antigen suspension with control sera; pool those with satisfactory reactivity, and test the pooled antigen suspensions with control sera.

(9) Mix the antigen suspension gently each time it is used. Do not mix the suspension by forcing back and forth through the syringe and needle, since this may cause the particles to break down which in turn may cause loss of reactivity.

Testing accuracy of delivery needles

(1) It is of primary importance to use the proper amounts of reagents, and for this reason the needles used should be checked every day. Practice will allow rapid delivery of antigen suspension and saline, but care should be exercised to obtain drops of uniform size.

(2) For the slide tests on serum, dispense the antigen suspension from a syringe fitted with an 18-gauge (1.2 mm) needle without bevel which will deliver 60 ± 2 drops of antigen suspension per ml when the syringe and needle are held vertically.

(3) Adjust the needles not meeting these specifications such that they deliver the correct volume.

Preliminary testing of antigen suspension

(1) Perform a VDRL qualitative test on the control sera of graded reactivity as described below.

(2) Reactions with control sera should reproduce the established reactivity pattern. The nonreactive serum should show complete dispersion of antigen particles.

(3) Do not use an unsatisfactory antigen suspension or pool of antigen suspensions.

Note: Control sera of graded reactivity (reactive, weakly reactive, and nonreactive) should always be included during a testing period to ensure the proper reactivity of the antigen suspension at the time the tests are performed.

Preparation of patient's serum

(1) Heat the clear serum obtained from centrifuged clotted blood in a water-bath at 56 °C for 30 minutes before testing.

(2) Examine all sera after removing them from the water-bath and recentrifuge those found to contain particulate debris.

(3) Reheat the sera to be tested at 56 °C for 10 minutes; this reheating should be done at least 4 hours after the original heating period.

(4) The sera must be at room temperature at the time of the test.

VDRL slide qualitative test on serum

Slide flocculation tests for syphilis are affected by room temperature. For reliable and reproducible results, tests should be performed within the temperature range of 23–29 °C. At lower temperatures, test reactivity is decreased; at higher temperatures, test reactivity is increased.

(1) Pipette 0.05 ml of heated serum into one ring of a paraffin-ringed or ceramic-ringed slide. (Note that glass slides with concavities, wells, or glass rings are not recommended for this test.)

(2) Add 1 drop (1/60 ml) of the antigen suspension to each serum with an 18-gauge (1.2 mm) needle and a syringe.

(3) Rotate the slides for 4 minutes; mechanical rotators that circumscribe a circle 1.9 cm in diameter should be set at 180 r/min.

Note: When this test is performed in a dry climate, slides may be covered with a moisture chamber containing a moistened blotter during rotation to prevent excessive evaporation.

(4) Read the test results under a microscope with a 10 × eyepiece and a 10 × objective, immediately after rotation.

(5) Report the results as follows:

Reading	*Report*
Medium and large clumps	Reactive (R)
Small clumps	Weakly reactive (W)
No clumping or very slight roughness	Non-reactive (N)

(6) A prozone reaction is encountered occasionally. This type of reaction is demonstrated when complete or partial inhibition of reactivity occurs with undiluted serum and maximum reactivity is obtained only with diluted serum. This prozone phenomenon may be so pronounced that only a weakly reactive or "rough" non-reactive result is produced in the qualitative test by a serum that is strongly reactive when diluted. *It is therefore recommended that all sera producing weakly reactive or "rough" non-reactive results in the qualitative test should be retested using the quantitative procedure before a report of the VDRL slide test is submitted.* When a reactive result is obtained on any dilution of a serum that produced only a weakly reactive or "rough" non-reactive result before dilution, report the test as reactive and include the quantitative titre (see examples under step 9 of the next section).

VDRL slide quantitative test on serum

Test quantitatively, *to an endpoint titre,* all sera that produce reactive, weakly reactive, or "rough" non-reactive results in the qualitative VDRL slide test. The dilutions of the serum to be tested are: undiluted (1:1); 1:2; 1:4; 1:8; 1:16; 1:32. Since a test slide normally has 12 rings, two (1:1 through 1:32) or three (1:1 through 1:8) tests may be performed on one slide (see Annex 3, Fig. 1).

Annex 3, Fig. 1. Test slide showing distribution of serum dilutions for testing two sera simultaneously
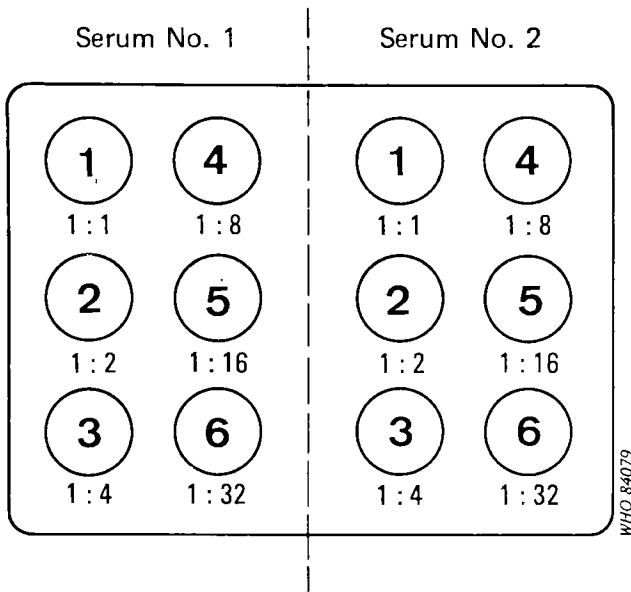
(1) Place the tubes of serum for quantification in a rack.

(2) Measure 0.05 ml of saline (9 g/l NaCl) into the 2nd, 3rd, and 4th paraffin rings in a row on the slide. Do not spread the saline. The saline may be delivered from an 18-gauge (1.2 mm) needle without bevel (0.025 ml per drop—use 2 drops), or a large needle, or calibrated dropper that delivers 0.05 ml in a single drop; these should be checked daily for accuracy of delivery.

(3) Using a safety pipetting device with a disposable tip that delivers 0.05 ml, measure 0.05 ml of serum into the 1st and 2nd rings. Avoid contamination of the instrument with serum.

(4) Using the same pipetting device and tip, mix the serum and saline in ring no. 2 (1 : 2 dilution) by drawing the mixture up and down in the tip 5–6 times. Avoid excess bubbles. (Use a clean plastic tip for each serum tested.)

(5) Transfer 0.05 ml of the 1 : 2 serum dilution to ring no. 3 and mix as described in step 4 to give a 1 : 4 dilution; transfer 0.05 ml of the 1 : 4 dilution to ring no. 4 and mix (1 : 8 dilution); discard 0.05 ml.

Additional serial dilutions may be set up for strongly reactive sera. (If the 0.05 ml of serum dilution has not spread within the entire area of a paraffin ring, spread it with the pipette tipe before proceeding to the next ring.)

(6) Add 1 drop (1/60 ml) of VDRL antigen suspension to each ring with an 18-gauge (1.2 mm) needle and syringe (as used for antigen suspension in the qualitative test).

(7) Rotate slides for 4 minutes. (Mechanical rotators that circumscribe a 1.9-cm diameter circle should be set at 180 r/min.)

Note: When tests are performed in a dry climate, slides may be covered with a moisture chamber during rotation to prevent excessive evaporation.

(8) Immediately after rotation, read tests microscopically with a 10 × ocular and a 10 × objective. Record the reading for each dilution tested.

(9) Report titre in terms of the greatest serum dilution that produces a reactive (not weakly reactive) result, in accordance with Annex 3, Table 1.

Annex 3, Table 1. Examples of results of VDRL slide quantitative test on serum[a]

Undiluted serum (1:1)	Serum dilutions					Result
	1:2	1:4	1:8	1:16	1:32	
R	W	N	N	N	N	Reactive, undiluted only, or 1 dilution
R	R	W	N	N	N	Reactive, 1:2 dilution, or 2 dilutions
R	R	R	W	N	N	Reactive, 1:4 dilution, or 4 dilutions
W	W	R	R	W	W	Reactive, 1:8 dilution, or 8 dilutions
N (rough)	W	R	R	R	N	Reactive, 1:16 dilution, or 16 dilutions
W	N	N	N	N	N	Weakly reactive, undiluted only, or 0 dilutions

[a] R = reactive; W = weakly reactive; N = non-reactive

ANNEX 4

Treatment schedules for venereal syphilis recommended by the WHO Expert Committee on Venereal Diseases and Treponematoses[a] (1984)

Stage of disease	Treatment		Post-treatment serological test
	Penicillin	Other[b]	
Early syphilis			
Primary, secondary and latent syphilis of less than 2 years' duration	Benzathine benzylpenicillin, 2.4×10^6 units as a single dose (1.2×10^6 units in each hip) or Aqueous procaine benzylpenicillin, 600 000 units daily for 10 consecutive days	Tetracycline hydrochloride, 500 mg by mouth, 4 times daily for 15 days or Erythromycin, 500 mg by mouth, 4 times daily for 15 days	3rd, 6th, and 12th months
Late syphilis			
Latent syphilis of more than 2 years' duration; late benign syphilis	Aqueous procaine benzylpenicillin, 600 000 units daily for 15 days or Benzathine benzylpenicillin, 2.4×10^6 units weekly for 3 successive weeks	Tetracycline hydrochloride, 500 mg by mouth, 4 times daily for 30 days or Erythromycin, 500 mg by mouth, 4 times daily for 30 days	Every 3 months during 1st year; every 6 months during 2nd year

Cardiovascular syphilis and neurosyphilis	Aqueous procaine benzylpenicillin, 600 000 units daily for 20 days[c]	As in latent syphilis	As in latent syphilis
Syphilis in pregnancy	As above, depending on stage of disease	Erythromycin by mouth, depending on stage of disease	Monthly during pregnancy, then as above depending on stage of disease
Congenital syphilis	In infants with abnormal cerebrospinal fluid: aqueous crystalline benzylpenicillin, 50 000 units/kg of body weight daily for 10 days In infants with normal cerebrospinal fluids: benzathine benzylpenicillin 50 000 units/kg of body weight in a single dose	Antibiotics other than penicillin are not recommended for infants with neonatal syphilis	Same as primary syphilis

[a] WHO Technical Report Series (Sixth report of the WHO Expert Committee on Venereal Diseases and Treponematoses) (in Press).
[b] Not indicated unless allergy to penicillin is clearly demonstrated.
[c] Failures can be treated with aqueous crystalline procaine benzylpenicillin, 4×10^6 units at intervals of 4 hours for at least 10 days.

Method for choosing a population sample

Introduction

It is not necessary to carry out a complete population survey in order to determine the prevalence of a disease in a given area. Valid information can be obtained by studying a sample of the population, provided that sampling is done with strict adherence to sound statistical methods. The reliability of the results will be affected by the choice of the sampling technique and the sample size.

When the disease in question is not uniformly distributed over an area, the first step is to divide that area into ecologically homogeneous zones. An ecologically homogeneous zone is an area in which the main factors having a bearing on the transmission of the disease (climate, topography, social and economic status of the population, access to health care facilities, etc.) are similar. This, however, does not mean that the prevalence of the disease will be the same or similar in all the villages in an ecologically homogeneous zone.

In epidemiological surveys, the selection of fixed-size clusters of households is a widely used procedure. Such clusters are representative of the entire population in terms of epidemiologically important variables such as age and sex distribution, educational level, family size, and economic status.

In principle, the total sample size (which is a multiple of the number of people in the fixed-size clusters) should be chosen according to the total population of the area being studied. In practice, however, the sample size will be restricted by the availability of resources (funds, personnel, equipment, etc.), logistic and other constraints, and the suspected prevalence of the disease—the lower the prevalence, the bigger the sample needed, and vice versa.[1]

In general, good results are obtained when the following simple rules are respected:

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[1] The determination of the optimum size and number of clusters required is a complex procedure, and it is advisable to consult a qualified statistician for this purpose.

— Choose the cluster size to correspond to the daily (or a multiple of the daily) work load of the team carrying out the survey. Depending on the size of the team, 100–200 individuals may be examined per day.
— Keep the cluster size small. It is desirable that the sample be spread out as much as possible over the total area to be investigated. In other words, it is better to have more clusters of smaller size.
— In dividing the area into ecologically homogeneous zones, use only the well established factors having a bearing on the transmission of the disease. Make independent estimates of prevalence for areas of high and low frequency of occurrence of the disease.

The sampling procedure

A multistage sampling technique is used for the selection of the total population sample. The first step involves the selection of a number of villages equal to the required number of clusters. Next, a predetermined number of individuals (these make up the cluster) is selected from each of these villages.

Selection of villages

The first step is to obtain an up-to-date list of all the smallest administrative units (villages, census areas, etc.) in the zone along with their corresponding census figures or estimated populations. In order to avoid bias in sampling and to select clusters randomly from the whole area, group the villages or census areas (or other administrative units) into larger areas (divisions, districts, etc.). Now follow the example given below.

Suppose that all the villages in an ecologically homogeneous zone fall into 6 divisions. (Urban areas are not included in such divisions because they are not considered to be ecologically homogeneous.) List the 6 divisions in any order along with their census figures or estimated populations. In a third column, cumulate the population figures as shown in Annex 5, Table 1.

At this stage it should be decided how many population clusters are required from each ecologically homogeneous zone. In our example, 10 such clusters are required from each zone. In order to select 10 villages from the 6 divisions in the zone, read 10 random numbers from Annex 5, Table 4 (or from any other table of random numbers). Since the total population of the zone in our example is in 6 digits, the random numbers should also be of 6 digits. Starting from the top left-hand corner of Table 4 and reading down the first column (and discarding the numbers

greater than 219 756, which is the total population of the zone) the following random numbers are obtained:

Number	Random number	Division identified by the random number
1.	103 748	D
2.	9 059	A
3.	107 313	D
4.	49 286	C
5.	17 623	A
6.	106 227	D
7.	66 364	C
8.	4 412	A
9.	153 062	E
10.	201 739	F

Each of these random numbers identifies the division from which the clusters should be picked. Note that more than one cluster may be picked from one division.

The identification of divisions from random numbers is quite simple. Since the first random number in our example (103 748) is greater than 87 295 (the cumulated population figure corresponding to division C in Annex 5, Table 1) and smaller than 119 059 (the cumulated population figure corresponding to division D in Annex 5, Table 1), it is considered as identifying division D—i.e., the division corresponding to the first number in the cumulated population column that is greater than the random number. Similarly, since the first number greater than 9059 (the next random number) in the cumulated population column in Annex 5, Table 1 is 24 125 (which corresponds to division A), it identifies division A. By applying the same procedure to the rest of the random numbers we get the following distribution of clusters within each division:

Division	Number of first-stage sampling units
A	3
B	–
C	2
D	3
E	1
F	1
Total	10

The next step is to identify the villages within the selected divisions from which the clusters will be picked. The procedure for this is the same as that for selecting divisions within an ecologically homogeneous zone. Suppose that division A (from which 3 villages have to be selected) has 32 villages. Now starting with any village, list these villages together with their populations and their cumulated populations as shown in

Annex 5, Table 1.   Cumulated population in an ecologically homogeneous zone by division

Division	Census figure or estimated population[b]	Cumulated population
A	24125	24125
B	17930	42055
C	45240	87295
D	31764	119059
E	58612	177671
F	42085	219756
Total	219756	—

[a] This may be an ecologically homogeneous zone or part of one.
[b] Excluding urban populations.

Table 2. Then place each of the random numbers that identified division A in front of the cumulated population figures to which they correspond—i.e., the first cumulated population figure greater than the random number. It can be seen from Annex 5, Table 2 that the random number 4412 corresponds to village no. 4, 9059 corresponds to village no. 8, and 17623 corresponds to village no. 16. Thus, villages 4, 8, and 16 are selected from division A.

Annex 5, Table 2.   The selection of villages from division A

Villages in division A	Population	Cumulated population	Random numbers[a]
1	1234	1234	
2	821	2055	
3	412	2467	
4	3214	5681	4412
5	619	6300	
6	1140	7440	
7	317	7757	
8	4272	12029	9059
9	528	12557	
10	614	13171	
11	803	13974	
12	1058	15032	
13	912	15944	
14	684	16628	
15	213	16841	
16	871	17712	17623
.	.	.	
.	.	.	
.	.	.	
32	1504	24125	
Total	24125	—	—

[a] These are the random numbers that identified division A arranged in rank order.

Since none of the random numbers that identified the divisions were between 24 126 and 42 055 (i.e., no random number identified division B), we can immediately proceed to division C. Again, starting with the cumulative population figure for divisions A plus B (42 055), make a list of all the villages in division C together with their populations and cumulated populations (see Annex 5, Table 3). By following the same procedure as for division A place the two random numbers that identified division C in front of the villages to which they correspond; those two villages are selected from division C. Repeat the same procedure for the rest of the divisions.

Annex 5, Table 3.   The selection of villages from division C

Villages in division C	Population	Cumulated population	Random numbers
		42 055	
1	1 765	43 820	
2	355	44 175	
3	3 848	48 023	
4	2 595	50 618	49 286
.	.	.	
and so on			

Selection of the population cluster

In epidemiological surveys it is advisable to have about 120 persons per cluster. The selection of the population is facilitated when a list of all the houses in the village being surveyed is available.

First, list all the houses serially. Then, select a random number from any table of random numbers such that it is not greater than the number of houses in the village. This random number will determine the house from which the survey should begin.

By dividing the total population of the village by the total number of houses, obtain the average number of individuals per household. This will indicate the number of houses that need to be surveyed. For example, if the average number of persons per household is 5, 24 houses will need to be surveyed in order to obtain a cluster of 120 individuals. Now, starting with the randomly selected house, survey the required number of houses serially. If the first house happens to have a serial number towards the end of the list, follow the serial order to the end of the list and then continue serially from house number 1.

If a house list is not available, the following procedure should be followed. When the village in question is small (of not more than 200 inhabitants), a map should be made showing all the houses in the village. The houses should then be numbered serially. For the sake of convenience it is better to number the houses in such a way that the first and the last house lie close to each other. Now repeat the procedure described above for a village where a house list is available.

If the village population is greater than 200 inhabitants, which is often the case, the following procedure should be followed. Divide the village into a few subareas on the basis of any naturally existing boundaries (e.g., there may be separate hamlets or it may be possible to divide parts of the village on the basis of roads or drains going through). Then make a list of the subareas, along with their estimated populations and cumulated populations. Pick a random number not greater than the total population of the village, and place it in front of the cumulated population figure to which it corresponds. The subarea corresponding to that cumulated population figure is selected. Map all the houses in that area and number them serially. Now follow the procedure described for the selection of a cluster in a small village.

Annex 5, Table 4. Table of random numbers

103 748	124 254	237 478	301 430	903 811	023 391
623 458	016 413	387 630	420 473	681 875	090 868
576 680	742 279	716 923	423 964	854 201	123 072
670 156	882 798	912 839	772 984	793 797	347 222
362 837	978 433	025 816	973 325	433 383	503 617
558 663	281 736	969 389	944 285	332 317	928 653
695 343	851 574	323 627	234 276	845 728	254 542
974 098	776 027	354 525	496 197	717 976	874 747
009 059	177 794	373 487	337 968	805 266	303 319
107 313	821 700	252 848	378 664	460 575	082 203
832 199	611 587	128 881	345 603	904 789	771 194
855 630	885 891	872 823	094 492	353 422	541 414
049 286	867 170	215 165	858 730	960 063	241 825
431 058	057 131	869 569	403 437	631 135	834 531
522 623	595 460	506 841	990 586	581 436	627 237
441 633	933 424	705 347	127 099	183 012	315 532
017 623	624 715	408 209	764 413	376 487	674 383
106 227	275 192	283 516	745 818	382 486	274 013
316 874	804 768	835 611	506 858	286 993	715 516
720 059	626 096	382 985	593 499	142 317	968 404
489 775	183 925	511 190	052 890	768 216	488 598
066 364	254 201	208 264	863 431	822 036	179 450
483 938	070 424	749 329	343 056	918 154	715 950
210 110	750 263	076 704	906 332	385 238	037 247
004 412	960 810	669 831	431 285	333 939	438 933
225 393	344 005	274 588	650 448	605 836	108 574
694 287	921 869	384 086	975 072	427 186	663 275
768 334	192 641	642 300	522 281	148 325	635 455
930 929	668 515	674 285	547 578	493 604	404 307
153 062	981 121	251 315	919 483	225 038	327 506
201 739	186 912	150 366	163 624	731 636	584 240
983 830	840 904	051 554	703 875	789 765	410 360
893 469	006 817	162 569	096 109	583 147	407 575
560 173	773 108	262 081	571 929	232 272	935 743
912 025	884 159	745 984	895 926	444 396	685 110