A GUIDE FOR EPIDEMIOLOGICAL STUDIES OF ORAL MANIFESTATIONS OF HIV INFECTION

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PREFACE

The International Collaborating Group on Oral Manifestations of HIV Infection was established in March 1988 as a forum for the development of collaborative activities in this area. It is composed of representatives of the Oral Health programme of the World Health Organization, Geneva, Switzerland; the Pan American Health Organization, Washington, DC, USA; the National Institute of Dental Research, National Institutes of Health, Bethesda, MD, USA; the Dental Disease Prevention Activity, Centers for Disease Control, Atlanta, GA, USA; the WHO Collaborating Centre on Oral Manifestations of HIV Infection, Copenhagen, Denmark; and the International Dental Federation (Fédération dentaire internationale, FDI), London, England.

In 1989, an FDI/WHO Joint Working Group on AIDS was formed to help implement some of the recommendations generated by the International Collaborating Group. It focused on four areas: health education and health promotion, aimed at both the public and the oral health profession; infection control; patient care; and epidemiology and surveillance.

As part of the activities relating to epidemiology and surveillance, the International Collaborating Group recommended the development of this guide, which is intended to provide a systematic approach to the design of epidemiological studies of oral conditions associated with human immunodeficiency virus (HIV) infection; to provide guidelines for the collection, management, analysis, reporting, and dissemination of data from these studies; and to facilitate the comparison of findings from different studies and different populations. It aims also to encourage oral health personnel, researchers, and public health practitioners to make oral health status an integral part of optimum case management and of surveillance activities of the diseases associated with HIV infection.

To achieve these aims, the publication provides:

- guidelines for implementing epidemiological studies of HIV-associated oral diseases;
- concise clinical diagnostic criteria for the major HIV-associated oral conditions;
- a standardized procedure for examining the head, neck, and oral cavity;
- an outline of suggested data collection variables;
- a guide for analysis and comparison of results for different populations;
- recommendations for reporting results.

Ideally, the guide should be included in epidemiological training activities, such as those of the Fogarty International Center's International Research and Awards Branch and of the National Institute of Dental Research. It builds upon the series of guides developed by the WHO Oral Health programme, especially the *Guide to epidemiology and diagnosis of oral mucosal diseases and conditions.*[1]

[1] World Health Organization. Guide to epidemiology and diagnosis of oral mucosal diseases and conditions. *Community dentistry and oral epidemiology*, 1980, **8**: 1–26.

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BACKGROUND

History

Within the past decade, HIV infection has assumed pandemic proportions. By 1992, 11 years after the acquired immunodeficiency syndrome (AIDS) was first reported, it was estimated that 11 million people of all ages worldwide had become HIV-infected. Many of these people have no signs or symptoms and are unaware that they are infected.

The infection is life-long and, once acquired, can be transmitted to others. Three modes of HIV transmission have been classified by WHO: sexual, parenteral (through direct inoculation of blood or blood products), and perinatal (from an infected woman to her fetus or infant, before, during, or after birth). As a result, the infants and sex partners of infected individuals, and people who share needles are at risk. People with haemophilia and other transfusion recipients are also at high risk in places where blood and blood products are not adequately screened for HIV.

Clinical symptoms appear as the virus destroys blood cells important for maintaining immunity. The most serious consequence of HIV infection is a decline in the number and function of the helper-inducer (T4, $CD4^+$) subset of lymphocytes. Progressive destruction of immune function allows the development of opportunistic infections and neoplasms, and leads finally to the full acquired immunodeficiency syndrome.

Despite progress in almost all aspects of HIV and AIDS research, understanding of the disease is still inadequate. Important questions remain about the determinants of individual susceptibility to the virus; factors that retard or accelerate disease progression; modes of entry into, and spread of the virus within, the body; interaction of the virus with various cell types; and reasons for the wide variety of immunological problems experienced by HIV-infected people. Knowledge of the modes of transmission and their relationship to the clinical course of the disease in different geographical locations is important in identifying behavioural and/or infectious co-factors that may be associated with the spread of HIV and the clinical course of the infection.

Oral lesions in HIV-infected individuals are frequent and varied (Table 1) and are among the first symptoms of infection. Moreover, the presence of pseudomembranous oral candidiasis and oral hairy

leukoplakia indicates a strong likelihood that the HIV infection is progressing towards AIDS. It is not surprising that early indicators of immunodeficiency occur in the oral cavity: concurrent immune suppression allows normally non-pathogenic microbes to proliferate, resulting in characteristic oral lesions.

Rationale

The objective of this guide is to lay the foundation for a standard system of designing epidemiological studies of HIV-associated oral diseases; of examining, identifying, and recording oral conditions that are — or may be — associated with HIV infection; and of analysing and interpreting study results. Conducting worldwide studies according to common principles will permit valid comparison of large quantities of data, and may well allow the identification of oral conditions with previously unsuspected links with HIV.

Comprehensive descriptions of the global spectrum of oral manifestations of HIV infection will come only from research in the

Table 1. Classification of oral lesions associated with HIV infection[1]

Group 1. Lesions strongly associated with HIV infection

candidiasis:
  erythematous
  hyperplastic
  pseudomembranous
Note: angular cheilitis is often associated with *Candida albicans.*
hairy leukoplakia
HIV-gingivitis
HIV-necrotizing gingivitis
HIV-periodontitis
Kaposi sarcoma
non-Hodgkin lymphoma

Group 2. Lesions less commonly associated with HIV infection

atypical ulceration
salivary gland diseases:
  dry mouth due to decreased salivary flow rate
  unilateral or bilateral swelling of major salivary glands
thrombocytopenic purpura
viral infections (other than Epstein-Barr virus[2]):
  cytomegalovirus[3]
  herpes simplex virus[4]
  human papilloma virus (warty-like lesions)
  - condyloma acuminatum
  - focal epithelial hyperplasia
  - verruca vulgaris
  varicella zoster virus[5]
  - zoster
  - varicella

Table 1 *(continued)*

Group 3. Lesions possibly associated with HIV infection

bacterial infections (excluding gingivitis/periodontitis):
- *Actinomyces israelii*
- *Enterobacter cloacae*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Mycobacterium avium intracellulare*
- *Mycobacterium tuberculosis*

cat-scratch disease
drug reactions (ulcerative, erythema multiforme, lichenoid)
exacerbation of atypical periodontitis
fungal infections other than candidiasis:
- *Aspergillus flavas*
- *Cryptococcus neoformans*
- *Geotrichum candidum*
- *Histoplasma capsulatum*
- Mucoraceae

melanotic hyperpigmentation
neurological disturbances:
- facial palsy
- trigeminal neuralgia

osteomyelitis
sinusitis
submandibular cellulitis
squamous cell carcinoma
toxic epidermolysis

[1] As agreed at a meeting of the EEC-clearinghouse on Oral Problems Related to HIV Infection, Amsterdam, 30–31 August 1990.
[2] human (gamma) herpesvirus 4
[3] human (beta) herpesvirus 5
[4] human (alpha) herpesvirus
[5] human (alpha) herpesvirus 3

greatest possible number of countries and cultures. Studies of this nature, and optimum comparability of their findings, are thus critically important.

This guide is intended for use by oral health practitioners who are not specialists in epidemiology and by epidemiologists who are interested in HIV-associated oral lesions but who may not be familiar with the full range of these disorders and the subtle distinctions between them. It emphasizes the need to adopt standardized procedures for investigation that reduce the chances of error in diagnoses based on clinical examinations alone.

Usage of terminology

Note: Terms printed in bold type in this and other sections of the guide are fully defined in the annex.

Epidemiological studies are concerned with the occurrence of disease or health conditions in human populations and usually fall into one of

two principal categories. In a **cross-sectional** or **prevalence study** every individual in the study group is examined once, at essentially the same time, in order to ascertain the **prevalence** of a particular disease or other condition. A **longitudinal** or **incidence study**, on the other hand, examines the population at specific intervals, over a period of time, in order to determine the **incidence** of a disease or condition, i.e. the number of new cases that occur in a defined population during the study period. Both types of study are **observational**, rather than experimental: nature is allowed to take its course and there is no intervention by the investigator.

Observational studies may be descriptive or analytical. A **descriptive study** is limited to describing the occurrence of a disease or condition in the study population, whereas an **analytical study** seeks to analyse the relationships between health status and other variables. This guide focuses on the development and implementation of descriptive studies.

The simplest epidemiological measurement is a count of the number of individuals presenting a given disease or condition at a given time. However, a more valuable indicator of health status may be obtained by dividing this count, or **numerator**, by the number of people at risk (which may be the entire study population) as the **denominator**; this yields a proportion known as the **prevalence rate**. Careful choice of an appropriate denominator allows valid comparisons to be made among and between different study populations. Prevalence rates are derived using cross-sectional study designs. **Incidence rates**, on the other hand, derive from longitudinal studies; the numerator is the number of new cases of a disease or condition arising during a specified period, and the denominator is the population at risk during this period.

Role of prevalence and incidence in epidemiological studies

In the particular case of an oral lesion associated with HIV infection, studies of prevalence are unlikely to reveal which condition preceded the other. Longitudinal studies of incidence rates, however, provide a better indication of the sequence of events and generally more information about the causes of disease. They also allow assessment of the risk — or likelihood — of an individual developing a specific disease or condition.

Unfortunately, there are many situations in which longitudinal studies are not feasible, because of the expense and/or difficulty of maintaining contact with the particular population under study. In these circumstances, investigations of HIV-associated oral conditions must rely on whatever information can be gleaned from prevalence studies of the conditions and of identified risk factors.

DESIGNING THE STUDY

Determining the objectives

The objectives of a study will determine the study design, the kinds of data to be collected and the form in which they will be recorded, and the sources of information. For instance, a study intended to estimate the proportion of a population suffering from a particular oral lesion should be designed as a cross-sectional — or prevalence — study. However, where the intention is to determine the number of individuals developing new oral lesions during a specified period, a longitudinal — or incidence — study would be necessary.

Although descriptive studies cannot be used to test hypotheses directly, their use may well shed light on assumed or expected associations. Complementary use of both types of study might be valuable, for instance, in testing whether the prevalence of oral lesions is different at different stages of HIV infection, and whether the incidence of specific oral lesions in HIV-infected individuals differs from that in uninfected people.

Appropriate areas of study, and even detailed objectives, may also be suggested by a review of the relevant literature. Whatever their rationale, it is essential that the objectives — and any underlying hypotheses — are fully established before detailed study design begins.

Selecting the study population

Types of population

The study objectives will determine the number and type of populations necessary. Appropriate populations for studies of the type addressed in this guide are those in which oral lesions are likely to exist or to develop; obvious examples are HIV-infected individuals and groups of patients attending oral care clinics. Studies of groups drawn from among the general public would be inefficient: both HIV infection and the oral lesions of interest are relatively uncommon, so that very large numbers of people would have to be examined in order to find any with both conditions. It is essential that the study population is clearly defined. Rules for inclusion should be clearly formulated and rigidly applied to all potential subjects.

It may not always be feasible to conduct large-scale epidemiological studies devoted solely to oral lesions, and it is therefore suggested that, wherever possible, oral conditions be studied in conjunction with other

clinical aspects of HIV infection. This has the advantage of making studies relatively inexpensive. However, cost is not the only factor in making this a rational approach: patients' medical histories and other clinical manifestations of HIV infection are critical in analysing oral findings.

Study and comparison groups

Use of a comparison group uninfected by HIV may not always be possible in an epidemiological study, but it is highly recommended. A comparison group is particularly important for studies of conditions such as periodontal diseases, which are common in the absence of HIV infection. Findings in the study population can then be compared with those in the comparison group, so that any increased risk of oral disease resulting from HIV infection can be estimated.

The selection of appropriate individuals for comparison is a critical component of study design and one that is influenced by the study objectives. Comparison groups should be as similar as possible to the infected population with respect to characteristics that may affect the prevalence or incidence of oral lesions, such as age, sex, race, ethnic group, health-related behaviour (including sexual practices and use of tobacco, alcohol, and drugs), and occupational factors. For example, if the study is designed to estimate the increased risk of oral candidiasis in HIV-infected individuals with haemophilia, the comparison group should consist of haemophiliacs who are not HIV-infected but who resemble the study group in all the other characteristics noted above.

As a general rule, the comparison group should contain at least as many individuals as the study group. The same diagnostic procedures and criteria should be applied to the examination of both groups. Ideally, the examiner should be unaware of the health status of any individual — in this particular case, unaware of whether an individual is HIV-infected or not — although this may not always be possible.

Selecting the sample size

The number of subjects who should be included in a study is an important consideration, and it is recommended that a biostatistician be consulted before the study is initiated. For cross-sectional studies, for example, it is essential to have an estimate of the proportion of the population likely to have the condition of interest, and of the magnitude of allowable error of the population mean. In some cases, an estimate may be available in the relevant literature, and may even be specific to the population to be studied.

In addition to the objectives of the study, the basic information required as a basis for calculating sample size is the following:

- the expected prevalence of the condition(s) being surveyed
- an estimate of the variance about the mean
- the required precision of the results
- the appropriate confidence level.

As shown in Table 2, sample sizes for different prevalence values vary considerably according to the required precision. Very large numbers of subjects are necessary if a precision level of ± 5% at the 95% confidence level is specified.

For a simple prevalence study of a population that has not previously been surveyed, the 95% confidence level — so often regarded as appropriate — may be too demanding.

When deciding on the type of study and the number of subjects to include, the investigator should also consider the facilities and resources that are available.

Developing data variables and recording forms

Data collection

It is essential that all data variables — that is, all the details that may be of relevance to the study — be identified at the design stage. A

Table 2. Sample sizes for different prevalence rates at two precision levels, ±5% and ±10%, for 95% confidence level

Note: It is assumed in this table that a simple random sample of individuals can be taken (rather than clusters such as households or villages).

Estimated prevalence in population, %	Sample size	
	Precision ± 5%	Precision ± 10%
2	80 000	20 000
3	45 000	10 000
4	35 000	9 000
5	30 000	7 000
6	25 000	6 000
7	20 000	5 000
8	20 000	4 500
9	15 000	4 000
10	15 000	4 000

thorough review of the existing literature may well be a useful adjunct to this process.

The first step is therefore to list all the information that must be gathered if the study objectives are to be thoroughly addressed, including not only the lesions of interest but also other medical, demographic, behavioural, and social factors relevant to the study population. Thus, in a study of oral lesions, information on tobacco use and poor oral hygiene is relevant, because both are **risk factors** for certain oral conditions.

It is sometimes the case that a risk factor is related to both an oral lesion and HIV infection; this is known as a **confounder** or **confounding variable**. When information on a suspected confounding variable is available, statistical methods exist for assessing its relative contribution to — in this case — any observed association between the oral lesion and HIV infection. When there is no such information, it may not be possible to characterize the true nature of the observed association. Examples of categories of variables are given in Table 3.

There are three basic sources of information on data variables: the individual's medical and/or oral health records; clinical examination of the individual; and questioning the individual. The methods used to

Table 3. Selected variables relevant to oral lesion status at time of examination

Demographic characteristics

Age
Sex
Race
Ethnic group
Country of residence
Occupation

Medical status

HIV status
Systemic disease
Current medications
Specific details — height, weight, history of tobacco use
T-cell levels (and/or other markers of immunodeficiency)

Other

If there is a focus on manifestations of HIV infection resulting from different modes of transmission, data on specific, related risk factors will be necessary

— sexual transmission
— blood products
— perinatal
— other

elicit information on each data variable from each source should be specified and recorded.

The second step is to develop a standardized form and/or questionnaire that will permit all information to be recorded in a clear and logical manner. Where possible, each variable noted as present in any individual should be quantified; there should also be provision for noting instances of an individual being unable to answer a particular question. The sequence of examination and/or questioning should be carefully planned to elicit the fullest possible information from each individual. It is also critical that the same sequence be used for every individual, and that data collection forms reflect this sequence, because variations in procedure may alter findings. In the clinical examination, for example, if the examiner conducts a gingival bleeding index *before* examining tissues for pathological changes, any changes are likely to be obscured by the bleeding.

The aim in developing data collection procedures and instruments is the gathering of reliable, valid, and practical information. The degree to which results obtained by a particular measurement procedure can be replicated is a measure of the **reliability** of the procedure, and the extent to which a measurement measures what it purports to measure is an expression of its **validity**. **Accuracy** is a reflection of both these qualities: it is the extent to which a measurement truly represents the attribute that is being measured.

Measurement of HIV infection status

A primary goal of any epidemiological study is to estimate as accurately as possible the **exposure** of interest — in this case, HIV infection status — as well as the outcome of interest. However, when the epidemiological survey is an adjunct to a much larger study of other aspects of HIV infection, its level of sophistication will be limited by that of the parent study. In such a case, the only available information on HIV infection status may come from surrogate measures, i.e. those based on medical history and clinical examination, and data of this type carry less weight than the results of laboratory investigations.

In all research reports, it is critical that the methods used to determine HIV status be clearly stated; as a minimum, these should ideally follow the guidelines for HIV sentinel surveillance recommended by the WHO Global Programme on AIDS.[1] A practical example of

[1] Slutkin G et al. *Sentinel surveillance for HIV infection: a method to monitor HIV infection trends in population groups*. Geneva, World Health Organization, 1988 (unpublished WHO document WHO/GPA/DIR/88.8, available on request from Global Programme on AIDS, World Health Organization, 1211 Geneva 27, Switzerland).

development of such instruments is provided by the WHO system for recording oral manifestations of HIV infection for those who are actually seropositive or are concerned that they may be. A package that provides a recording form, instructions for use, and diagnostic criteria — with photographs — is available from WHO.[1] HIV-antibody testing with licensed enzyme-linked immunosorbent assay (ELISA) kits is recommended. To be classed as HIV-infected, an individual must have repeated positive results in tests for the presence of HIV-antibody. If possible, a specimen that is HIV-positive in an ELISA should be retested by ELISA; results should then be confirmed by a further test, such as Western blot.

In countries where HIV-2 is known or suspected to exist, testing should be performed as recommended by WHO[2] — to the extent that available resources and reagents allow. Generally, the protocol is to use ELISA testing for both HIV-1 and HIV-2, with confirmation of positive results in either test by Western blot. Data on the route of virus transmission, duration of infection, and the individual's current clinical and immunological status should be collected whenever possible.

Diagnostic criteria

Although it is desirable to record all oral conditions detected in the course of the study, it may be more practical to target certain conditions that are expected to be the most prevalent. This section of the guide is concerned with the latter, although Table 1 lists all the oral lesions that have been reported in HIV-infected individuals.

Diagnosis of the more prevalent HIV-associated oral lesions may be based on the criteria outlined below, which are in turn adapted from criteria published by the EEC-clearinghouse on Oral Problems related to HIV Infection and the WHO Collaborating Centre on Oral Manifestations of the Human Immunodeficiency Virus,[3] and by the USA Oral AIDS Collaborative Group.[4] These criteria provide guidance for the establishment of presumptive diagnoses based solely

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[1] *WHO system for recording oral lesions possibly associated with HIV infection.* Available on request from Oral Health, World Health Organization, 1211 Geneva 27, Switzerland.

[2] *Operational characteristics of commercially available assays to determine antibodies to HIV-1 and/or HIV-2 in human sera.* Geneva, World Health Organization, 1991 (unpublished document GPA/RES/DIA/91.1, available on request from Global Programme on AIDS, World Health Organization, 1211 Geneva 27, Switzerland).

[3] EEC-clearinghouse on Oral Problems Related to HIV Infection and WHO Collaborating Centre on Oral Manifestations of the Human Immunodeficiency Virus. An update of the classification and diagnostic criteria of oral lesions in HIV infection. *Journal of oral pathology and medicine*, 1991, **20**(3): 97–100.

[4] Greenspan JS et al. Oral manifestations of HIV infection. Definitions, diagnostic criteria, and principles of therapy. The USA Oral AIDS Collaborative Group. *Oral surgery, oral medicine and oral pathology*, 1992, **73**(2): 142–144.

on clinical manifestations. Additional procedures must be followed for more substantive diagnoses, and notes on these are included below where appropriate.

Fungal diseases

pseudomembranous candidiasis	Yellow-white, loosely adherent (wipable) plaque, located anywhere in the mouth. Removal leaves erythematous mucosa, with or without bleeding.
erythematous (atrophic) candidiasis	Erythematous (atrophic) macular patches on mucosal surfaces. Areas such as dorsum of tongue, which normally have papillae, are often depapillated. Palatal mucosa is usually affected at the same time. Colour ranges from light pink to scarlet.
angular cheilitis	Fissures or linear ulcers at corner of mouth. Varying degrees of inflammatory erythema. Hyperkeratosis may be present peripheral to the fissure.

Note: Definitive diagnosis of the above lesions includes the positive morphological verification of candidal hyphae in a smear of the lesion, including potassium hydroxide, periodic acid-Schiff-stained, or Gram-stained preparations.

Bacterial diseases

erythematous gingival banding	A continuous band of erythema at the gingival margin, at least 1 mm in width, extending across the entire tooth surface. Adjacent teeth are also often affected.
necrotizing gingivitis	Ulcerative or necrotic destruction of the gingival tissues, often with blunting or cratering of the interdental papilla. Pseudomembrane formation may also be present. *Tissue destruction is limited to gingival tissues and does not involve alveolar bone.*
necrotizing periodontitis	Advanced necrotic destruction of the periodontium with rapid loss of periodontal attachment and alveolar bone. Necrotic bone fragments may be visibly exposed. *Note*: Definitive diagnosis includes rapid tissue loss, within 4 weeks, and exclusion of other causes of periodontal soft and hard tissue destruction. Radiographs are required.

chronic periodontitis	Any destructive periodontal disease with bone loss, pocket formation, or tooth mobility, but *not displaying signs of ulceration, necrosis, or pseudomembrane formation.* The condition may or may not be HIV-related.

Viral diseases

Herpes simplex[1] *infection*

recurrent herpes labialis	Single or multiple vesicles or ulcers with crusting on the vermilion portion of the lips and adjacent facial skin. (The recurrent form may be more severe, extensive, and persistent in the HIV-infected patient.)
herpetic stomatitis	Solitary, multiple, or confluent lesions that may be noted together with vesicles on keratinized mucosa, including hard palate, attached gingiva, and dorsum of the tongue. Occasionally, non-keratinized mucosa may be involved. Round to slightly irregular margins with minimal to no erythematous halos are present.

Note: Definitive diagnosis of the herpes simplex virus infections includes demonstration of the virus by the use of tests such as immunohistochemical analysis and culture.

Epstein-Barr virus[2] *infections*

hairy leukoplakia	A vertically corrugated, slightly elevated white surface alteration of the lateral or ventral tongue margin, which does not wipe off. May also be seen at other oral sites, usually in conjunction with tongue lesions.

Note: Confirmation of the presence of herpes-type viral particles by electron microscopy or demonstration of Epstein-Barr virus by in situ hybridization techniques is required for definitive diagnosis of this lesion. Treatment by antifungal drugs may differentiate this lesion from chronic hyperplastic candidiasis.

Idiopathic conditions

recurrent aphthae	Single or multiple recurrent, well circumscribed oral mucosal ulcers with a whitish fibrinous pseudomembrane surrounded by an erythematous halo. Usually limited to non-attached mucosa but may extend to tissue overlying periosteum in infected patients.

[1] Human (alpha) herpesvirus 1.
[2] Human (gamma) herpesvirus 4.

atypical ulcerations	These lesions may appear in any location of the oral mucosa. They are usually deep, crateriform, and covered by fibrin.

Neoplasms

oral Kaposi sarcoma	One or more erythematous, slightly bluish or violaceous macules or swellings, with or without ulceration. Predominantly seen on the palate or the gingiva.
non-Hodgkin lymphoma	A firm, elastic, often somewhat reddish or purplish swelling, with or without ulceration. The gingiva and the palatal mucosa are the sites of preference.

Note: Examination of biopsied tissue is the only way to obtain a definitive diagnosis of these lesions.

Clinical examination procedures

Although adequate examination of the oral cavity requires little equipment, good lighting is essential: a dental light, headlight, or head mirror would be suitable. Retraction of the cheeks and tongue requires mouth mirror(s) or tongue blade(s), and a dental mirror is necessary to visualize some areas of the oral cavity and oropharynx. If the appropriate equipment is available, photographs should be taken of all interesting or unusual oral conditions encountered during the survey. These are extremely useful in cases of doubtful diagnosis; for follow-up and comparisons; for documentation; and as a supplement to drawings/diagrams and written descriptions. A library of photographs illustrating the different conditions diagnosed in the local population is always a valuable addition to the usual teaching and training materials.

A methodical procedure should be adopted for oral examination and consistently applied. Patients' dentures should be removed before examination begins. Changes such as red and/or white discoloration, ulcers, and lumps or swellings should be documented. Tissue texture should be observed, and lesions should be examined by digital palpation. All necessary infection control precautions should be observed. The following procedure is recommended:

Lips

Examine the lips with the patient's mouth closed and again with the mouth open. Note the colour, texture, and any abnormalities of the vermilion border.

Lower labial mucosa and sulcus

Visually examine the mandibular vestibule with the patient's mouth partially open. Observe the colour and any swelling of the vestibular mucosa and gingiva.

Upper labial mucosa and sulcus

Visually examine the maxillary vestibule and frenulum with the patient's mouth partially open.

Commissures, buccal mucosa, and buccal sulcus (upper and lower)

Using retractors, and with the patient's mouth wide open, examine the entire buccal mucosa, extending from the commissures back to the anterior tonsillar pillar. Note pigmentation, colour, texture, and mobility of the mucosa. Make sure that the commissures are fully examined, and not covered during retraction of the cheek.

Gingiva and alveolar ridges (processes)

Check from all sides (i.e. buccally, palatally, lingually).

Tongue

With the patient's mouth partially open and the tongue at rest, inspect the dorsum of the tongue for any swelling, ulceration, coating, or variation in size, colour, or texture. Note also any abnormalities in the pattern of papillae covering the surface of the tongue. Ask the patient to protrude the tongue, and note any abnormality of mobility. Using retraction, inspect the margins of the tongue, then observe the ventral surface. The tongue — and particularly the posterior lateral margins — can be examined more efficiently by grasping the tip with a piece of gauze to assist full protrusion, but *it is essential to observe adequate infection control measures.*

Floor of the mouth

With the patient's tongue elevated, inspect the floor of the mouth for abnormalities. Use a mouth mirror to push the tongue from side to side so that the tissues lining the lingual sulcus and the retromylohyoid fossa can be inspected.

Hard and soft palate

With the patient's head tilted backwards and the mouth wide open, gently depress the base of the tongue with a mouth mirror. Inspect first the hard palate and then the soft.

Note: Some patients have a sensitive gag reflex.

All mucosal and facial tissues, the tongue, and the floor of the mouth, including the submandibular and cervical lymph nodes, should be

palpated. The neck and submental areas should be palpated to determine the condition of the lymph nodes (enlarged, movable/fixed, tender/non-tender). Examination of the oropharynx requires the patient's mouth to be wide enough open for the tonsillar tissue and upper pharynx to be visible; gentle depression of the dorsal tongue may facilitate this procedure.

Training research personnel

Epidemiological studies require the accurate and consistent recording of all variables of interest and the application of clearly formulated diagnostic criteria. The consistency of diagnosis of oral lesions depends in turn on appropriate training of clinical examiners and, particularly, on standardization of the procedures they follow. The objectives of this standardization are:

- to ensure uniform interpretation and application of diagnostic criteria to the diseases and conditions observed; and
- to ensure a consistent standard of examination among different examiners and thus minimum variation in results.

Proper training is critical for all personnel involved in an epidemiological study, including health staff who conduct interviews and clinical examinations, those who record the findings, those who undertake laboratory analyses, and those responsible for reporting results. The training required will vary from study to study, and even from country to country, but each study should be preceded by a formal training session to review and standardize the procedures that are to be used.

IMPLEMENTING THE STUDY

Pretesting and pilot-testing

Pretesting data collection forms and protocols for oral examination — generally by using family members and professional colleagues — provides information that is important to the development and improvement of study instruments. The next step is pilot-testing, which represents the first attempt to implement the study in the field. Using small numbers of readily available individuals who are typical of the eventual study population, pilot-testing provides the opportunity for refining data collection techniques and eliminating inconsistencies.

Operation and monitoring

The collection, recording, and coordination of data should be monitored throughout the study, and this requires the application of strict quality-control measures, established at the study design stage. These measures include the following:

- review and editing of all questionnaires, data collection forms, and logbooks for accuracy, completeness, consistency, legibility, correct use of subject identifiers and entry codes;
- periodic recalibration of examiners to maintain consistency;
- replication of laboratory tests to allow periodic checks on the comparability of results obtained;
- mechanisms to safeguard the integrity and confidentiality of data;
- review of data collection procedures to ensure adherence to the study protocol.

REVIEW AND ANALYSIS OF RESULTS

Data preparation

Before analysis, data need to be reviewed and edited to identify coding errors and missing information; incomplete records should be omitted from analysis. Moreover, the type of analysis that is possible will depend on the quality and completeness of the data.

Data analysis

Table 4 provides examples of basic data layouts for cross-sectional and longitudinal studies: the examples assume that information on an appropriate comparison group has been gathered.

Hypothetical example:

Oral lesions were studied in a group of individuals attending a clinic for treatment of sexually transmitted diseases. The total number of individuals available for examination was 1000, of whom 50% were HIV-infected. All were examined every 3 months for a period of 1 year. Results were as follows:

HIV status	No. of individuals with newly identified lesions:				
	Exam 1	Exam 2	Exam 3	Exam 4	Total
Infected ($n = 500$)	5	2	0	3	10
Uninfected[a] ($n = 500$)	2	0	2	1	5
Total	7	2	2	4	15

[a]Some initially uninfected individuals may seroconvert during the study.

a. Cross-sectional approach — prevalence (see Table 4*a*)

At examination 1, prevalence was calculated as follows:

HIV status	No. of individuals with oral lesions:		Total
	present	absent	
Infected	5	495	500
Uninfected	2	498	500
Total	7	993	1000

Prevalence of oral lesions in HIV-infected individuals = 5/500 = 0.01 = 1%
Prevalence of oral lesions in uninfected individuals = 2/500 = 0.004 = 0.4%.

b. Longitudinal approach—incidence rate (see Table 4*b*)

Lesions identified at examination 1 were assumed to be new and not present before the start of the study; they were therefore included in the calculations. (Pre-existing lesions observed on later occasions must be omitted from calculations.)

HIV status	No. with oral lesions after 1 year	Person-time
Infected	10	500 persons × 1 year = 500 person-years
Uninfected	5	500 persons × 1 year = 500 person-years

Incidence rate for oral lesions in HIV-infected individuals
= (10 new cases/500 persons) × 1 year
= 0.02 new cases per person-year

Incidence rate for oral lesions in uninfected individuals
= (5 new cases/500 persons) × 1 year
= 0.01 new cases per person-year

The ratio of incidence rates in HIV-infected individuals to that in uninfected individuals shows that new oral lesions were twice as common in HIV-infected individuals as in uninfected individuals (0.02/0.01 = 2).

Table 4. Data layout for epidemiological studies of oral lesions associated with HIV infection

a. Cross-sectional approach (prevalence)

HIV status	No. of individuals with oral lesions: present	absent	Total
Infected	a	c	$a + c$
Uninfected	b	d	$b + d$
Total	$a + b$	$c + d$	$a + b + c + d$

Total study population

$= (a + b + c + d)$

Prevalence of oral lesions in HIV-infected individuals

$= [a/(a + c)] \times 100\%$

Prevalence of oral lesions in uninfected individuals

$= [b/(b + d)] \times 100\%$

b. Longitudinal approach (incidence rate)

HIV status	No. of individuals with oral lesions present	absent	Person-time
Infected	a	c	$(a + c)$ × duration of follow-up
Uninfected	b	d	$(b + d)$ × duration of follow-up
Total	$a + b$	$c + d$	

Incidence rate for new oral lesions in HIV-infected individuals (new cases per person-time)

$= [a/(a + c)] \times$ follow-up time (in years)

Incidence rate for oral lesions in uninfected individuals (new cases per person-time)

$= [b/(b + d)] \times$ follow-up time (in years)

PREPARATION OF REPORTS

The amount of detail to be included in a report will depend on the intended use of the data. For instance, data for publication in a scientific journal should be more concise than those that will be the subject of a monograph. However, the report should have the same general form in all cases and should contain the following elements.

Introduction

The introduction should include a description of the problem and any relevant background information.

Statement of purposes of study

This statement should include a succinct but clear description of the aims of the study, and expectations of how the results will be used.

Materials and methods

It is customary to include the following in this section:

- *General description of the methods*, including the population and the geographical area covered by the study.
- *Nature of the information collected*, including both general data and the diagnostic criteria applied to the specific diseases or conditions recorded.
- *Methods of collecting data.* Description of methods such as questionnaire, interview, clinical examination.
- *Subject selection.* The criteria for selection of study subjects should be defined. Selection problems should be reported, and numbers and descriptions of eligible individuals who were not examined should be included. In longitudinal studies, the numbers of subjects lost to follow-up, the times at which these losses occurred, and the reasons for loss should be reported.
- *Physical arrangements and details of personnel.* The physical arrangements made for examination, the equipment used, and details of oral examination methods should be included. The organization, training, and experience of all personnel involved in collecting and processing data should be noted.
- *Statistical analysis.* Brief descriptions of statistical methods applied to raw data should be included, and any non-standard procedures should be described with appropriate references.

Results

The presentation of results will depend on their intended use. Where brevity is important, only the more important results should be described. Summary tables may be included in the main text of the report or collected in a separate appendix. Table 4 provides an example of a summary table. Graphs, histograms, scatter diagrams, etc. may be used to illustrate points that are neither easily described in the text nor readily apparent from tables. All figures and tables should be fully and unambiguously labelled so that their understanding does not require reference to the text.

Discussion and conclusions

The discussion should be used to explain the extent to which the study fulfilled its objectives, to consider possible errors and limitations in the data, to highlight results of particular interest and consider their significance, and to compare results with other relevant published information. The conclusions may include suggestions for further research or for application of findings to health services and planning.

Summary

A summary of the report should be provided, and is frequently required to be of a length suitable for use as an abstract. It should include the objectives of the study, the number of subjects examined, the most important results, and any unusual or unexpected findings.

References

A list of publications mentioned in the report should be included.

SELECTED FURTHER READING

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Annex

DEFINITIONS OF EPIDEMIOLOGICAL TERMS[1]

accuracy — The degree to which a measurement, or an estimate based on measurements, represents the true value of the attribute that is being measured.

analytical study — A study designed to examine associations, commonly putative or hypothesized causal relationships. An analytical study is usually concerned with identifying or measuring the effects of risk factors, or is concerned with the health effects of specific exposure(s).

confounding variable — (Synonym: **confounder**) A variable that can cause or prevent the outcome of interest, is not an intermediate variable, and is not associated with the factor under investigation. Such a variable must be controlled in order to obtain an undistorted estimate of the effect of the study factor on risk.

cohort study — See **longitudinal study**

cross-sectional study — **(Synonym: prevalence study)** A study that examines the relationship between diseases (or other health-related characteristics) and other variables of interest as they exist in a defined population at one particular time. The presence or absence of disease and the presence or absence of the other variables (or, if they are quantitative, their level) are determined in each member of the study population or in a representative sample at one particular time.

denominator — The lower portion of a fraction used to calculate a rate or a ratio. The population (or population experience, as in person-years, passenger-miles, etc.) at risk in the calculation of a rate or a ratio.

descriptive study — A study concerned with and designed only to describe the existing distribution of variables, without regard to causal or other hypotheses. An example is a community health survey, used to determine the health status of the people in a community.

[1] The definitions in this annex are taken, with minor modifications, from: Last JM, ed. *A dictionary of epidemiology*, 2nd ed. New York, Oxford University Press, 1988, with the permission of the publisher.

exposure

1. Proximity and/or contact with a source of a disease agent in such a manner that effective transmission of the agent or harmful effects of the agent may occur.
2. The amount of a factor to which a group or individual was exposed; sometimes contrasted with dose, the amount that enters or interacts with the organism.

incidence

The number of instances of illness commencing, or of persons falling ill, during a given period in a specified population. More generally, the number of new events, e.g. new cases of a disease in a defined population, within a specified period of time.

incidence rate

The rate at which new events occur in a population. The numerator is the number of new events that occur in a defined period; the denominator is the population at risk of experiencing the event during this period, sometimes expressed as person-time.

longitudinal study

(Synonyms: **cohort study, incidence study**) The method of epidemiological study in which subsets of a defined population can be identified who are, have been, or in the future may be exposed or not exposed, or exposed in different degrees, to a factor or factors hypothesized to influence the probability of occurrence of a given disease or other outcome. An essential feature of the method is observation of the population for a sufficient number of person-years to generate reliable incidence or mortality rates in the population subsets.

numerator

The upper portion of a fraction used to calculate a rate or a ratio.

observational study

Epidemiological study in situations where nature is allowed to take its course; changes or differences in one characteristic are studied in relation to changes or differences in other(s), without the intervention of the investigator.

prevalence

The number of instances of a given disease or other conditions in a given population at a designated time. When used without qualification, the term usually refers to the situation at a specified point in time (point prevalence).

prevalence rate (ratio)

The total number of all individuals who have an attribute or a disease at a particular time (or during a particular period) divided by the

population at risk of having the attribute or disease at that point in time or midway through the period.

prevalence study — See **cross-sectional study**.

relative risk

1. The ratio of the risk of disease or death among the exposed to the risk among the unexposed.
2. Alternatively, the ratio of the cumulative incidence rate in the exposed to the cumulative incidence rate in the unexposed.

reliability — The degree of stability exhibited when a measurement is repeated under identical conditions. Reliability refers to the degree to which the results obtained by a measurement procedure can be replicated. Lack of reliability may arise from divergences between observers or instruments of measurement or instability of the attribute being measured.

validity (of a study) — The degree to which the inferences drawn from a study, especially generalizations extending beyond the study sample, are warranted when account is taken of the study methods, the representativeness of the study sample, and the nature of the population from which it is drawn.