Risk assessment on yellow fever virus circulation in endemic countries

Working document from an informal consultation of experts

A Protocol for risk assessment at the field level

World Health Organization

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Pandemic and epidemic diseases publications are available online at www.who.int/csr/resources/publications.

Abbreviations and acronyms

CAR	Central African Republic
CIESIN	Center for International Earth Science Information Network
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunization
ERI	WHO Epidemic Readiness and Intervention Team
GAVI	the GAVI Alliance (Global Alliance for Vaccines and Immunization)
GIS	geographical information system
IgG	immunoglobulin G
IgM	immunoglobulin M
IHC	immunohistochemistry
MOH	Ministry of Health
PCR	polymerase chain reaction
PRNT	plaque-reduction neutralization testing
RA	risk assessment
WHO	World Health Organization
YF	yellow fever
YFV RNA	yellow fever virus ribonucleic acid
YFV	yellow fever virus

Introduction

In 2006, the Global Alliance for Vaccines and Immunization (GAVI) Board approved the Yellow Fever Investment Case, Its goal was to prevent yellow fever (YF) epidemics and provide a healthy global YF vaccine market. As part of this initiative, an innovative strategy to control and reduce the risk of YF outbreaks was implemented, which consisted of two components:

- inclusion of YF vaccine in routine immunization programmes for 9-month-old infants;
- implementation of preventive mass vaccination campaigns to increase the population's immunity in high-risk areas rapidly, and to protect older age groups susceptible to contracting YF disease.

Twelve of the highest risk YF-endemic countries in Africa were selected for preventive mass vaccination campaigns.

In September 2006, a panel discussion of YF experts in Dakar, Senegal, identified the most pertinent risk factors related to YF in the African Region. Out of the 40 risk factors listed, only six were identified as important according to the availability and regularity of data provided by the countries:

- districts situated in an ecological risk zone (between 15°N and 10°S)
- districts that have been reporting confirmed YF cases since 1960
- districts that have been reporting suspected YF cases since 1960
- number of years in which YF cases have been reported since 1960
- districts neighbouring a district that have been reporting YF cases since 1960
- proportion of the population in the district that is not immunized.

Since 2008, an increase in YF virus (YFV) circulation has been reported in Africa (Central African Republic [CAR] and Cameroon) and the Americas (Argentina, Brazil, Colombia, Venezuela, and Trinidad and Tobago). Therefore, a group of YF experts developed a multidisciplinary risk assessment (RA) tool, which includes serosurveys in human and non-human primates, and assessment of vector density and infectivity. Ecological and environmental indicators are also included in the RA to attempt to explain why the changes have occurred and, potentially, to predict future risk. The RA tool was first used in CAR where multiple outbreaks had been reported in a short period of time in different areas of the country. The main objective was to assess the perceived increase of YFV circulation in the previous years and in areas that had been historically "silent". The RA tool provides information about ecological areas at risk in zones where YFV is circulating – it is not adequate for demonstrating the absence of YFV circulation in a cross-sectional study. Nevertheless, it could be adapted if at least two studies could be done in a specific area at an interval of 5–7 years, which represents the cycle of YFV circulation.

The methodology proposed in this document aims at supporting a more systematic approach for data collection and analysis to assess YFV risk in an area, and to determine appropriate vaccination strategies. As more assessments and data are available, attempts will be made to establish criteria to compare the level of risk for YFV transmission in the areas studied. These criteria are likely to better inform future developments, such as mathematical modelling, or to support other tools in decision-making.

Under the Yellow Fever Initiative, the World Health Organization and key partners will assist Member States to adapt and use this YF RA tool to make relevant decisions on YF vaccine policy and protect their populations from the disease.

This assessment tool will be completed by a quantitative scoring method that will help the decision-making process at national level.

This working document was initially implemented in Cameroon and in the Central African Republic. It was then improved and validated during an informal consultation of yellow fever experts on 13-14 September 2011 in Geneva, Switzerland.

1 Purpose

The purpose of this working document is to provide guidance on the methodology to be used for assessing the yellow fever (YF) virus circulation, and the epidemic potential in endemic countries or areas where the YF epidemic risk is unknown or has recently changed. It is not a prescriptive document, and the methodology may be adapted based on the purpose and objectives of the risk assessment.

One of the main objectives is to gather sound data that can be used to develop detailed recommendations regarding the use of YF vaccines in the countries (e.g. the need for either preventive or reactive campaigns, or the use of the vaccine in the childhood expanded programme on immunizations). These data can be obtained through evaluating human and non-human primate, vectors, and environmental and ecological variables as described in more detail throughout the document (see Annex 1 for more information on YF).

This document describes the RA methodology for YF virus circulation in areas at risk, and is primarily intended for public health specialists.

Participants

The Informal Consultation on Risk Assessment of Yellow Fever virus Circulation, which took place in WHO HQ from 13 to 14 September 2011, brought together a group of experts whose objective was to assess the risk of yellow fever and improve and validate a general protocol based on Central African Republic and Cameroon experience. The experts were selected based on their expertise in the domain of yellow fever; most of them had published research articles and had a broad academic background related to the subject discussed.

The members of the Informal Consultation completed the standard WHO form for declaration of interests prior to the meeting. All participants were requested to confirm their interests, and to provide any additional information, relevant to the subject matter of the meeting. These were then reviewed by the WHO Secretariat.

Several participants described academic interest in the subject matter of the meeting, including participation in non-commercially funded clinical studies. These were not regarded as conflicts of interest since they formed the basis of the expertise of the panel.

Only one participant declared an interest with a potential conflict with the objectives of the meeting; Thomas P. Monath, who is employed by Kleiner Perkins Caufield and Byers. On further review, it was adjudged that Dr Monath's employment status did not pose any conflict with the specific objectives of this meeting and did not warrant his exclusion from any part of the proceedings.

In accordance with WHO policy for the conduct of the meeting, the participants provided working papers or advice to the meeting and partook in discussions, but did not participate in the decision-making process of the meeting and made no contribution to the subsequent writing and finalization of the guidelines.

2 Risk assessment methodology

This risk assessment (RA) methodology aims to translate a complex, multidimensional reality into a simple framework to facilitate decision-making for yellow fever (YF) control.

This section presents an RA methodology and describes the recommended processes that concern all factors, including selection of sample sites, human resources needs to conduct field assessments, collection and specimens testing, and determining logistics and cold chain requirements. These factors are discussed for the assessment of YF virus (YFV) activity among human populations, non-human primates and vectors. Ecological factors are also considered.

The rationale for this methodology differs from the approach used by the Yellow Fever Initiative in 2006. At that time, historical data of several YF events in the 12 most endemic countries in Africa were analysed through mathematical modelling to prioritize the implementation of mass vaccination campaigns.

The current RA methodology was designed for use mainly in middle- and low-endemic areas. It aims to provide a cross-sectional assessment of the epidemic risk – particularly in areas where there is limited historical information or data on YFV activity.

This document describes the methodology and activities to be conducted during the multidisciplinary field missions. During the RA process, multidisciplinary teams will be sent to the selected urban and rural population centres within each of the identified ecological zones. At a minimum, the teams should consist of:

- entomologists
- epidemiologists
- virologists/laboratory personnel
- veterinarians or veterinary technicians (when necessary).

Other professionals such as government representatives, qualified technicians, local guides, logisticians and drivers will complete the team, and provide local support to maximize the efficiency of the field mission.

In addition to the local RA team, an ecological expert will assess and choose the indicators linked to the changes and migrations in the area, such as vegetation coverage, rainfall levels and the evolution of “green areas” in previous years (e.g. forest fires, deforestation, disordered/recent human settlements).

The recommended approach includes the systematic collection of serological samples of humans and non-human primates, as well as the capture of mosquitoes. In addition, data related to ecological factors that are considered to be linked to the risk of viral transmission are used to help define the ecological zones for sampling and interpreting the results. This approach aims to ensure a rapid and simple, yet cost-effective, field mission that maintains a high level of scientific rigour. The practical approach is based on the following considerations:

- **Rapidity:** the duration of the field mission should take approximately two to three weeks, but will depend on the organization and the coordination of the mission;
- **Scientific method:** the exercise should use preliminary analysis and local experiences;
- **Simplicity:** the implementation of the approach should take into account the specificities of the local environment and capacities.

Ideally, this approach should be standardized to facilitate future comparisons between areas, African and American countries, and urban and rural contexts.

Ministry of Health services and experts should be responsible for training in-country personnel to assist in the RA process and ensure that the following activities are considered:

- collection of administrative authorizations and ethical clearance from the national authorities;
- recruitment of professionals familiar with blood laboratory techniques for the human and non-human primate serosurveys, and/or virus isolation and detection;
- review of instructions regarding sterile blood drawing technique, such as the type of blood tube to be used and volumes to be obtained;
- review of appropriate personnel protected measures;
- review of laboratory testing techniques for YF, with laboratory staff at regional reference laboratories.

A complete checklist and an example programme are available in Annex 2.

Although a large part of the training will take place in a structured classroom setting, a field briefing should be performed when the team and experts arrive to coordinate the work and to ensure the efficiency of the field mission. Personnel will be provided additional and/or refresher information while in the field as needed.

2.1 Selecting sample sites[26]

The main objective is to obtain representative data from a variety of locations, which can then be extrapolated to larger areas. This approach requires some degree of statistical rigour, using randomization when possible; however, this needs to be weighed against the practicality of implementing the study in the field.

A multistage cluster design is the recommended approach to determine the sites and the populations to be included in the assessment.

The RA methodology selects the sites for sampling in three stages:

1. identification of the ecological zones
2. selection of the locations within an ecological zone
   and
3. selection of the closest urban and rural population centres.

2.1.1 Stage 1: Identification of the ecological zones

The identification of the distinct ecological zones should be based, at minimum, on rainfall and vegetation coverage. This information is used to account for differences in humidity, temperature and land cover – factors that indirectly influence the density of mosquitoes and, thus, may impact YFV activity and circulation among human and non-human primates. See Section 3 of the protocol for more information on how to establish ecological zones.

2.1.2 Stage 2: Selection of the locations within an ecological zone

After mapping the distinct ecological zones for each area of interest, points to be sampled within each ecological zone should be randomly selected.

It is recommended that at least two random points are generated per zone to ensure a sufficient level of variability and to decrease the likelihood of selecting one area that may not be representative of the entire zone. More than two sampling locations should be considered if the zone is large or the density of the population is high. The feasibility of visiting all the sites within a reasonable time frame, however, has to be considered when determining the number of sampling sites per zone.

Several computer programs are available that can assist in randomly selecting points within a zone (e.g. polygons). This approach is encouraged as opposed to using more subjective approaches (e.g. asking local personnel where there are places that are easy to get to and have people who may participate), which will decrease the representativeness of the data collected.

2.1.3 Stage 3: Selection of the closest urban and rural population centres

Using the geographical coordinates (latitude and longitude) of each randomly selected point, the urban and rural population centres closest to that point should be identified. The rationale for sampling both an urban and rural area is that mosquito and non-human primate densities may vary between urban and rural locations. For instance, urban centres may have *Aedes aegypti* or transitional zone mosquitoes and fewer non-human primates compared with rural areas where *A. africanus* or *Haemogogus* spp. mosquitoes and more primates are present.

Local personnel familiar with the area should be consulted when selecting urban and rural population centres based on the population size and the ecosystem of the area. The random points can often be plotted using free programs and tools such as Google Earth™, which can help locate the selected points on a map.

If the randomly selected point does not coincide with a specific population centre, it is then recommended to choose the closest centre to that point within the ecological zone for sampling.

2.2 Assessing the human population

The objective of this section is to determine the level of the YFV circulation within a given at risk population. This section provides details on the target population, calculation of the human sample size, sampling methods, inclusion and exclusion criteria, and potential limitations.

The level of YFV circulation is measured by collecting and analysing human blood specimens. This method is used to estimate the prevalence of naturally-acquired YFV-specific antibodies within a given population. The primary factors that may impact the analysis of human blood specimens are:

- Yellow fever vaccine coverage. The currently available assays cannot differentiate between YF antibodies developed against the wild virus (natural immunity) or the vaccine virus (acquired immunity). Because of this, serosurveys have less utility in places with high vaccine coverage. Therefore, sampling should either be avoided in places with high vaccine coverage or the sample size needs to be calculated to compensate for this – usually by increasing the sample size.
- Cross-reactions with other known flaviviruses. Cross-reactivity between flaviviruses can occur in currently available assays, including the more specific plaque-reduction neutralization test (PRNT). Cross-reactive antibodies are more likely to occur in the elderly because they have a higher probability of contact with multiple flaviviruses in their life compared with children, who are less likely to have been exposed. Given this, including children in the study is essential to assess YFV risk in a population.

2.2.1 Target population

Blood specimens should be taken from individuals who are at least 9 months old. Younger infants may have maternal immunoglobulin G (IgG) antibodies present in their blood. However, as mentioned, children should be included in the assessment, when possible, to ensure that individuals with less potential for flaviviral cross-reactive antibodies in their serum sample are a part of the study.

2.2.2 Sample size calculation[24]

The sampling approach described is a multistage cluster design. This approach assumes that ecological zones are uniform in their risk for YFV activity and that any point in that zone would be representative of the whole zone. Thus, a randomly selected point can be used to extrapolate to the rest of the zone.

To determine the sample size for each randomly selected point, the following information is needed:

- population of the ecological zone
- estimate of YF vaccination coverage by age and ecological zone
- estimated rates of non-response or non-participation, based on similar surveys (roughly 15–30%)
- population of the randomly selected urban and rural population centres.

The population estimate of the zone, YF vaccination coverage and non-response rates should be determined before field deployment. If not known before deployment, the population of the randomly selected urban and rural population centres can be obtained by the team afterwards.

There are three steps to calculating the sample size.

Step 1: Determine the initial sample size

This can be done by calculating the baseline rate of YFV-specific antibodies in the population due to vaccination. This step is needed as the sample size should be adjusted in order to detect an increase in antibody rate above the rate due to vaccination. Below is an example on how to calculate the YF vaccination antibody rate in a population.

Box 2.1 Calculating baseline vaccination antibody rates in a population

Formula

a) Determine the number of persons < 5 years:
# of persons ≤ 5 years within the ecological zone = population in ecological zone × % of population ≤ 5 years

b) Persons with yellow fever (YF) antibodies acquired due to vaccination:
# of persons with YF antibodies due to vaccination = # of persons ≤ 5 years within the ecological zone × average Expanded Programme on Immunization (EPI) coverage per year

c) Percentage of population expected to have YF antibodies due to vaccination:
% of population expected to have YF antibodies due to vaccination = # of persons with YF antibodies due to vaccination / # of persons ≤ 5 years in the ecological zone

Baseline data

Population in the ecologic zone = 100 000 persons

Average EPI coverage per year = 72%

Number of years the EPI programme has been in place = 5 years

Proportion of population ≤ 5 years = 10%

Example calculation of acquired immunity in the zone due to vaccination

100 000 persons × 10% = 10 000 persons ≤ 5 years

10 000 × 72% = 7200 persons with YF antibodies due to vaccination

7200 / 100 000 = 7.2% of the population is expected to have YF antibodies due to vaccination

Note: Some estimate of variance should be made around the estimate (e.g. 7.2% ± 3%).

Step 2: Estimate the sample size after adjusting for clustering and non-response/non-participation

Clustering may occur and, therefore, sampling should be done at the household level. Household members will be more likely to have similar activities and behaviours, such as going to get water or to work. This may skew the representativeness of the results and therefore should be included in the sample size adjustment (see Box 2.2).

Box 2.2 Adjusting the sampling when the household size is large

When the household size is large, the sampling should be adjusted. A minimum design effect of 2 should be used; it means that the number of households needs to be multiplied at least by 2, avoiding that, in a given location, too few houses are sampled. A large design effect should be considered to control for potential clustering and to ensure that the sampled households are representative of the characteristics of the zone. The initial sample size should then be multiplied by the design effect.

Non-response or non-participation should be compensated by increasing the sample size. Ideally, households and participants within a household who do not wish to participate should not be replaced. The rationale for this is that the characteristics of the randomly selected persons might be influenced by their availability. For example, young men tend to travel to a neighbouring area to work during the day. These young men may have different exposures that might increase or decrease their risk of acquiring YF disease. If only persons who are at home during the day are selected, it may underrepresent young men in the sampled population, and under- or overestimate the risk of YF for the population. Estimates of 15–30% of non-response have been used in previous RAs. The proportion of non-response or non-participation should be used to increase the final estimate by that proportion.

Table 2.1 demonstrates estimated sample size calculations with the various adjustments that are needed to derive a final adjusted sample size population, by zone.

Table 2.1 Estimated sample size calculations

Population size	Estimated YF-vaccinated population (95% CI)[a]	Initial sample size	Design effect adjustment (2)	Oversampling adjustment (15%)	Adjusted sample size for zone
500	5 ±3% 10 ±15% 15 ±7%	144 108 83	288 216 166	86 65 50	374 281 216
1000	5 ±3% 10 ±15% 15 ±7%	169 121 91	338 242 182	101 73 55	439 315 237
1500	5 ±3% 10 ±15% 15 ±7%	179 127 94	358 254 188	107 76 56	465 330 244
3000	5 ±3% 10 ±15% 15 ±7%	190 132 97	380 264 194	114 79 58	494 343 252
10 000	5 ±3% 10 ±15% 15 ±7%	199 136 99	398 272 198	119 82 59	517 354 257
100 000	5 ±3% 10 ±15% 15 ±7%	202 138 100	404 276 200	121 83 60	525 359 260

CI, confidence interval; YF, yellow fever. [a] *To be determined from Expanded Programme on Immunization data and population distribution.*

Step 3: Determine the number of persons to sample in a given centre

Once the final sample size estimate per zone is calculated (Step 2), divide the final sample size estimate per zone by the number of randomly selected points per zone.

To determine the number of persons to sample in a given urban and rural centre, the population size of those locations should be obtained. (If this is not available before field deployment, this calculation can be done once in the field. See Box 2.3 for an example on how to calculate the final sample size of the randomly selected urban and rural locations.)

Box 2.3 Sample size calculation to determine final sample sizes for randomly selected points

Formula

1. Determine the number of selected points
   # per randomly selected points = Sample size per zone / random points per zone
2. Determine the % population in centre
   % of population in centre = population size of centre X / total population of zone X
3. Sample size for zone X
   Sample size for zone X = # per randomly selected points × % of population in centre

Baseline data

Random points per zone = 2

Sample size per zone = 400

Zone A

Population of urban centre A = 2850
Population of rural centre A = 150
Total population zone A = 3000

Zone B

Population of urban centre B = 10 154
Population of rural centre B = 749
Total population zone B = 10 903

Calculation of number of persons to sample for selected towns and villages

400/2 = 200 per randomly selected points

Zone A

2850 / 3000 = 95% of population in urban centre
150 / 3000 = 5% of population in rural centre

Sample size for zone A

95% × 200 = 190 persons
5% × 200 = 10 persons

Zone B

10 154 / 10 903 = 93% of population in urban centre
749 / 10 903 = 7% of population in rural centre

Sample size for zone B

93% × 200 = 186 persons
7% × 200 = 14 persons

2.2.3 Sampling in the field

The following steps describe a process for field sampling:

1. **Communicate with officials and health-care workers.** Upon arrival in the community, a communicator within the field team should provide a message to the community official and health-care workers that describes the trial and provides more information on YF disease and prevention.
2. **Determine the approximate number of households in each town and village, and the average number of people per household.** Information should be obtained from local officials.
3. **Determine the number of households to sample.** This is calculated by taking the calculated sample size divided by the estimated average number of people per household.
4. **Determine which households to visit.** Once the number of households is calculated, a random number table can be used to determine which households are to be visited. Households should not be replaced (e.g. do not replace the household if, after repetitive attempts to visit it, there were no occupants in the household). See the human sampling size calculation in Box 2.3 for more information.
5. **Invite all members (≥ 9 months of age) of the randomly selected household to participate.** The study objectives are explained and information is given to the household on YF disease and prevention. Afterwards, consent should be obtained from adults and from the parents of minors within the household (assent of children may also be necessary depending on local practices). Either written or oral consent should be obtained, depending on the literacy rate of the population and local practices. Persons should be informed that the samples are collected using numbers to protect their identity, and therefore the results cannot be linked back to any one person.
6. **Obtain standard demographic data.** On consenting individuals, standard information on demographics (age and sex) and YF vaccination status (year and documentation of vaccination) should be collected, and then a blood sample taken.
7. **Draw blood.** Blood is obtained in serum separator tubes from each consenting participant by age stratification:
    a. Children from 9 months to 10 years old (3 ml)
    b. Adults aged 10 years and older (5–10 ml).

Note: If, while drawing blood, it becomes clear that someone in the household is unwell, the person should be advised to go to the closest health centre and, if feasible, the field team should assist in this arrangement and alert local health-care staff of the situation. If the person is suspected of having YF based on signs and symptoms, then an investigation form is filled out by local health personnel and an outbreak investigation launched if the blood sample testing confirms YF infection (the location is identified retrospectively by household).

8. **Store all samples in ice boxes and separate serum within 24 hours.**
9. **Assign each participant a unique, unidentifiable study number.** Each study participant is assigned a study number and the corresponding blood sample should only be labelled with the study number and date the sample was obtained. Information obtained about the participant including YF vaccination status, age, sex, and geographical location should be recorded and linked to the study number. There should be no link between any participant and their sample to ensure patient confidentiality.

Alternative sampling methodology

The sampling approach described in Section 2.3 is robust, and the randomization of households may be challenging based on the details available for that location. Given this, alternative sampling methodologies can be considered as long as the impact of selecting a different approach is discussed and does not negatively impact the scientific validity of the study.[17]

One additional consideration in determining the sampling methodology is whether or not the human, mosquito and potential non-human sampling will occur at the same randomly selected points. If sampling is conducted in the same location, resources (e.g. financial and human) can be pooled together. However, the disadvantage of this approach is that the entomology team will require more time to complete their sampling than the human serosurvey team. Therefore, the entomology team may prevent the human serosurvey team from relocating if they complete their assessment, and mosquito sampling should be done during the rainy season, which may not correspond with the time that other teams are deployed to the field. The data collected will tell a more complete story of what was happening in the location.

Alternatively, if human and mosquito sampling is decoupled, this could allow more points to be selected for the human sampling. This approach will most likely increase the time needed to obtain results and resources needed to conduct several separate assessments. However, this might decrease the variance of the final estimate.

Inclusion and exclusion criteria

Inclusion

All persons ≥ 9 months old living in the sample areas.

Exclusion

Populations living in areas where YF vaccine campaigns were recently conducted.[a]

Limitations[24]

There are limitations to using this type of sampling approach:

- Due to safety concerns in certain regions, sampling may not be conducted completely randomly, and can lead to sampling bias and influence the final results.
- Inaccurate estimates of population and vaccine coverage can lead to errors on the sample size calculation, and affect the quality of the observations and the analysis of the RA.

2.3 Assessing non-human primates

The objective is to assess the non-human primate populations capable of amplifying YFV and the extent of YFV circulation within that population. In this section, the RA methodology provides details on the target population, sampling methods, inclusion and exclusion criteria, and potential limitations.

The role of non-human primates in YF epidemiology in Africa and the Americas is very different. In the Americas, sylvatic YF often occurs in unvaccinated people working in the jungles or sylvatic environment, and in the nearby areas. In sylvatic enzootic areas, monkey deaths due to YFV often precede human cases. For this reason, surveillance systems to detect deaths of non-human primates are often put in place. When there is a cluster of non-human primate deaths, blood samples are taken and tested for YFV infection. The cases are then reported to the health system authorities and preventive vaccination activities in people may be undertaken.

In Africa, non-human primate species usually do not die from YFV infection and instead acquire immunity against YF when they are infected. Therefore, surveillance systems to detect deaths of non-human primates are generally not effective.

2.3.1 Target population[26]

Blood specimens of non-human primates should be safely collected for the detection of YFV-specific antibodies. For health and ethical reasons, non-human primates that have died or been killed (for reasons other than the study) should be chosen for sampling. Using dead non-human primates will reduce the risk of possible accidents that may occur with live or sedated animals.

If live animals are used for sampling, it is then recommended to trap, sample, mark and release them back into the same ecosystem.

2.3.2 Non-human primate sampling methods

Basic information about the non-human primates sampled will be kept in a line list, including sample number, species, age and location. Non-human primates should not be killed for the specific purpose of the study and some species are excluded according to the exclusion criteria.

The natural parks personnel and local guides may be able to provide relevant observations on non-human primate populations for sampling at the randomly selected sites.

[a] *If a population is believed to have reliable data on YF vaccination status (e.g. presence of yellow cards or good immunization registries), persons with a documented history of YF vaccination could be excluded. In this case, blood samples would be taken from all persons who had no history of vaccination, if randomly selected, but not from persons who were already vaccinated before. This would alter how the initial sample size was determined, but would also minimize any confusion of YF antibodies due to natural exposure to YFV or to the vaccine virus. In previous RAs, data on YF vaccination status were not readily available.*

Blood samples will be taken from non-human primates that have died or been killed for reasons beyond the assessment to determine the proportion of animals that are seropositive for YFV-specific antibodies. Dead primates should be sampled only when the central team is in the location (estimated to be up to four days). Tissue samples (e.g. liver, kidney) may be obtained for histological and virological purposes (e.g. PCR, immunohistochemistry [IHC], virus isolation) to confirm that the non-human primate was infected with YFV.

The recommended method of sampling is convenience sampling. This is recommended due to the difficulty in determining the location and population density of non-human primates, as well as the safety and animal protection regulations for trapping and sampling live animals.[24] Convenience sampling is primarily recommended when there are minimal surveillance systems, and the population density, age and species of non-human primate population is unknown. Convenience sampling can provide information regarding the degree of exposure to the YF within a particular location; however, results cannot be extrapolated to the rest of the zone. Given this, the utility of non-human primates sampling needs to be weighed against the resources and potential risks of sampling.

Other methods of sampling include:

- **Random sampling,** This method is recommended when data – such as species type, absolute number of susceptible species and age distribution – are within a sampling location, such as a national park. These data would be more informative and could be used to state more definitive conclusions about risk.
- **Longitudinal sampling.** This method is recommended in areas where there is high vaccination coverage in the human population. In the Americas, this method is more informative.

2.3.3 Inclusion and exclusion criteria

Inclusion[26]

All YFV-susceptible non-human primate species living in the randomly selected sampling location. A list of non-human primate species susceptible to YFV infection is provided in Annexes 3 and 5.

In Africa, non-human primates less than 2 years old should be sampled to detect recent virus circulation.

Exclusion

Non-human primates no more than 2 years old in Africa.

In the Americas, it is not easy to determine the age of small monkeys such as marmosets and tamarins (Cebidae and Callithricidae).

2.3.4 Limitations[24]

The convenience sampling method for the non-human primates may result in low numbers of samples due to the availability of non-human primate corpses and/or a lack of random sampling for multiple locations (e.g. in deep forests). Therefore, the results obtained should not be used to infer the level of YFV antibodies for other non-human primates throughout the same ecological zone.

To use the random sampling method, good information on the non-human primates population and age distribution are required. To use the longitudinal sampling, the population density needs to be known, and can be used in the Americas.

2.4 Assessing vectors

The objective of vector surveys is to assess the various types of vectors present in a specific ecological zone, as well as to determine whether YFV is present in any of the species. In this section, the RA methodology provides details on the target population, sampling methods, inclusion and exclusion criteria, and potential limitations.

The ecological zones used for vector surveys should be determined mainly by rainfall and vegetation coverage. This information can be obtained at the country level or analysed from satellite photos. Each zone has unique features that will affect the types and abundance of mosquitoes. It is important to note that susceptible mosquito species remain infected throughout their life and can transmit the virus transovarially.

2.4.1 Target population[26]

Adult and larval mosquitoes should be collected at each randomly selected point within the corresponding urban or rural location, and the surrounding forests.

2.4.2 Mosquito sampling methods[24]

Both larval and adult mosquito sampling is conducted at each of the randomly selected sites. Timing of the sampling should be considered, because vector abundance will depend on rainfall. Thus, sampling during or at the end of the rainy season will provide a more accurate depiction of risk. If there is more than one rainy season, sampling should be done at a time when cases of YF have historically been diagnosed, to improve the likelihood of recovering YFV from the mosquitoes.

Another consideration for mosquito sampling is the minimum time needed at each location to adequately sample the mosquito population. If the mosquito sampling is conducted in combination with human and/or non-human primates, detailed plans need to be discussed before field deployment to determine the feasibility of this approach.

Larvae and pupae sampling

In domestic and peridomestic environments, collections are made in randomly selected houses, and indoor and outdoor areas inhabited by humans. Sampling should be conducted simultaneously with the human study and should cover all households visited by the epidemiological team.

The sample size should be estimated according to a random sampling method (cluster analysis). The larval samples should be collected at the same randomly selected households (and surrounding areas) in which the serosurveys are being conducted.

Artificial and natural mosquito breeding sites should be inspected. Each container holding both potable and non-potable water should also be inspected. Containers with at least one larva or pupa of *A. aegypti* are considered to be positive for mosquito larvae. Larval and pupae samples should be collected randomly from infested containers and sent to the laboratory (mosquito insectary) for rearing and identification of the emerging adult stages. Samples tested in the laboratory will provide an estimation of the population, sex ratio and the density of mosquito females.

Adult stages sampling

Since alternative methods do not exist for an efficient collection of YF vectors (particularly the sylvatic vectors), adult mosquitoes are sampled by human landing capture.

Individuals participating in human landing captures are vaccinated against YFV and are given malaria chemoprophylaxis. If reliable alternative methods for sampling adult mosquitoes become available, these techniques should be used.

When sampling, it is recommended to:

- preserve external key morphological characters in order to allow precise species identification;
- keep mosquitoes alive until the laboratory procedures by
  - providing them with a 10% sucrose solution
  - transporting the cages in ice boxes.

Africa

Adult mosquito samplings should be performed inside and outside human dwellings, and in the neighbouring forests between 16:00 and 20:00 (the peak activity time for both domestic and sylvatic vectors). Mosquitoes should be captured live and transferred to cages. Female gravid traps should be set up in domestic and forest environments to increase the chances of capturing mosquitoes.

Americas

Adult mosquito samplings should be performed in endemic areas. *Haemagogus* spp., *Sabethes* spp. and other potential YF vectors may be very active all day long, especially around midday. Please note that the specific timing for sampling is less important than in Africa

In certain circumstances, insecticide spray collection and/or aspiration of resting females are performed in randomly selected dwellings.

2.4.3 Mosquito classification

Larvae and pupae collected in the field should be reared for at least for 4–6 days to evaluate the emerging adults accurately. Mosquito species are identified in the adult stage using a stereoscopic microscope.

Adults are pooled according to their geographical origin, sex and species (maximum of 10 mosquitoes per pool) and then stored at –70 °C or in liquid nitrogen until testing of relevant species can be performed. Adult mosquitoes will be identified and sorted in a monospecific batch, and tested for the presence of YFV by real time PCR.

A table of African vectors is provided in Annex 4 and South American vectors in Annex 6.

Table 2.2 Mosquito sampling methods by location

Domestic	Extra domestic/sylvatic/rural
Vector density • Breteau and Container indices • Human landing collection	Vector identification • Human landing collection
Vector competence • Aspiration	Vector competence • Aspiration
Vector identification • Human landing collection	Human-biting frequency • Human landing collection
Circulation • Human landing collection	Circulation • Human landing collection

2.4.4 Inclusion and exclusion criteria

Inclusion[26]

All competent mosquito species able to transmit the YFV within the same area.

Exclusion

All mosquito species not competent at transmitting YFV. See table in Annexes 4 and 6.

2.4.5 Limitations[24]

There are challenges in trapping the sylvatic mosquito species that tend to live high in the forest canopy. Furthermore, this can complicate the ability to determine sample size calculations.

Currently, human landing capture studies are the most appropriate method to obtain adult mosquitoes capable of transmitting YFV. However, this technique does present a risk to persons involved in the capture study. The risks can be mitigated by the use of the YFV vaccine and having antimalarial treatments available. If reliable alternative methods for sampling adult mosquitoes become available, these techniques should be used.

If sampling during the rainy season cannot occur (i.e. sampling is taking place during the dry season), the density of mosquitoes sampled will likely be affected negatively. In this case, ovitraps may be used to detect and monitor indirectly the mosquito frequency.

2.5 Specimen testing logistics and cold chain requirements

Blood samples (human and non-human primates) and mosquitoes captured during the field mission must be transferred under appropriate conditions to the laboratories for testing. To ensure the best quality of the testing and results, it is important to consider the following recommendations for serosurvey and mosquito sampling.

2.5.1 Serosurvey sampling

Human and non-human blood samples are collected in serum separator tubes. The serum is separated and transferred to a cryovial, and the clotted blood components are disposed of as biohazard waste. The serum is stored at 4 °C for no longer than two days before the sample is frozen at –70 °C or tested at the laboratory. Samples are maintained and shipped with standard cold boxes and ice packs.

All serum specimens are tested for YFV-specific immunoglobulin M (IgM) and IgG antibodies using enzyme-linked immunosorbent assay (ELISA). All samples testing positive for YFV-IgM or IgG are then analysed for neutralizing antibody to YFV by PRNT.

When possible, the ELISA test should be conducted at a national laboratory within the same country. Serum samples that are not tested at a country's national laboratory need to be frozen at –70 °C and shipped to a regional WHO Collaborating Centre for YF.

Given the potential for cross-reactive antibodies within the flaviviral genus, any sample which that tests positive for YFV-IgM or IgG should be assessed for antibodies against other flaviviruses known to occur in the area, such as West Nile virus, Zika virus or dengue virus. These tests should be conducted in a national laboratory when possible, or frozen and shipped to a facility with ELISA capabilities. If any of these assays are seropositive by ELISA, then a more specific plaque-reduction neutralization test (PRNT) will be performed.

The samples should be tested in batches. A laboratory testing algorithm and a guideline for interpreting laboratory results are provided in Annex 7.

2.5.2 Non-human primates sampling

In addition to potential serosurveys (see testing methods in the previous section), consider collecting tissue samples, such as from the liver and/or kidney, from dead non-human primates (e.g. monkeys). In the Americas, a necropsy is performed after death. Monkeys found dead in the forest or in the surrounding areas should be sampled. The detection of YFV by IHC and the presence of histopathological signs for YF need to be assessed. Collecting tissues is often not practical in the typical field assessment, because it is conducted over a short time period and may be unlikely to yield sufficient numbers of dead animals.

2.5.3 Mosquito sampling

When testing mosquitoes for YFV, specimens belonging to the same species should be identified on a chill table and grouped into batches of no more than 10 mosquitoes. They should then be ground and centrifuged. The supernatant collected should be used to both extract YFV RNA (to be detected by real time PCR) and to isolate the virus (through cell culture inoculation).

3 Assessing ecological and environmental factors

Ecological and environmental factors can provide complementary information for risk assessments (RAs). Access to data and information on the environmental and demographic situation in the area of interest, both historically and at the time of the study, may support the interpretation and analyses of yellow fever virus (YFV) activity, and offer potential relevant information for future RAs.

The use of ecological and environmental indicators serves to support the RA of yellow fever (YF) outbreak at the study time by:

- informing the initial identification of ecological zones for sampling sites;
- supporting the interpretation of the RA results by helping explain how and why ecological and environmental conditions influence the changes in the vector, human and non-human primate populations;
- informing future work on modelling and prediction on YFV activity.

Some indicators – such as land use, and industry development and population movement – provide information on the interactions between the human, vector and non-human primate populations. It is important to consider in further detail which ecological and environmental indicators are most informative and for what purpose, as outlined in Table 3.1.

Table 3.1 Ecological and environment indicators that potentially impact YFV activity

Indicator	Relevance	Source of information
Temperature: maximum, minimum, average Time period: 60 years or, at the very least, the past 10 years; at a resolution of 30 metres or less	Vegetation type and food availability for non-human primate populations Migration of reservoir and humans Range of mosquito habitat Promoting transmission by shortening the virus incubation period	World Meteorological Organization National Meteorological Services of the country concerned
Elevation:[b] if temperature data are limited or non-existent, elevation can be used instead	Influence on the reach of mosquito habitat is 2300 m altitude	GIS HealthMapper
Rainfall: maximum, minimum, average; spatial distribution over time Time period: several decades; and need to consider start and length of the dry and wet seasons	As above	International Research Institute (Columbia, USA) Generate rainfall time series for the region of concern
Vegetation coverage Time period: at a minimum, the past 10 years	Forest cover change during the past 10 years, such as deforestation, land use changes, crop changes, rural vs urban area change Changes in the range of mosquito habitat	National archives
Land use and industry Time period: at a minimum, the past 10 years	To better understand human demographic changes in the region and accompanying effects, such as tourism, mining, oil exploration and timber	Country agencies, ministry of energy, trade, mining, etc.
Population movement[c] Time period: at a minimum, the past 10 years	Migration, tourism, and trade in the region and across borders	Center for International Earth Science Information Network

GIS = geographic information system

[b] *Use of elevation to delineate areas with risk. YF risk area is delineated for second administrative subdivisions, based on evidence of YF infection in human and in non-human primates.*

[c] *Direct or indirect measures of migration (human or non-human primates) are assessed, when possible, and could include information about recent industry and human presence (e.g. oil company usage, mine exploitation, wood exploitation, tourism activity), and human migration for economical or work reasons. Indirect complementary mapping information about vector evolution in the past 10 years, that includes the incidence of malaria cases by health district, could be evaluated.*

3.1 Ecological zones – sample site selection[24]

A multistage cluster design is used to select sampling sites for the YF RA. Although this can be a complex design in which two or more levels of units are embedded in each other, it can lead to a more cost-effective field mission.

The sampling design includes two stages:

1. Distinct ecological zones within the country are defined based, at a minimum, on vegetation coverage data and rainfall patterns (see Figure 3.1 for an example). These indicators are important, because mosquito type and density are linked closely to vegetation and rainfall. A polygon is constructed around each ecological zone to define its limits.
2. Within each polygon, a minimum of two points are selected using a random point generator. Each point should be defined by a set of coordinates (latitude and longitude), and represents the location of the urban and rural centres to be included for sampling in the field mission (see Figure 3.2 for an example).

Figure 3.1

Two ecological zones in southwestern Cameroon based on rainfall and vegetation data

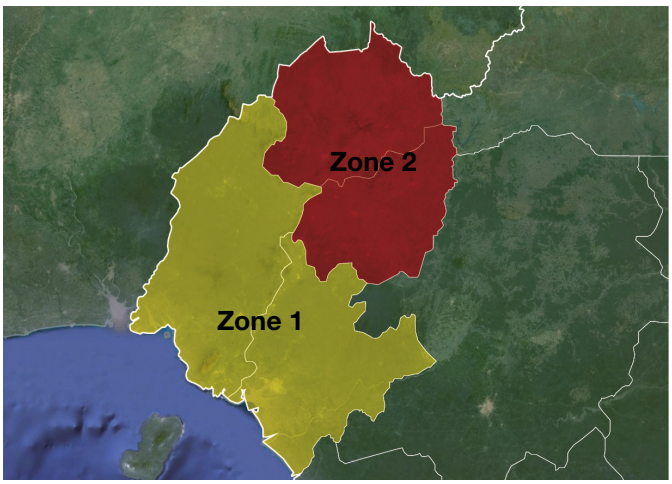
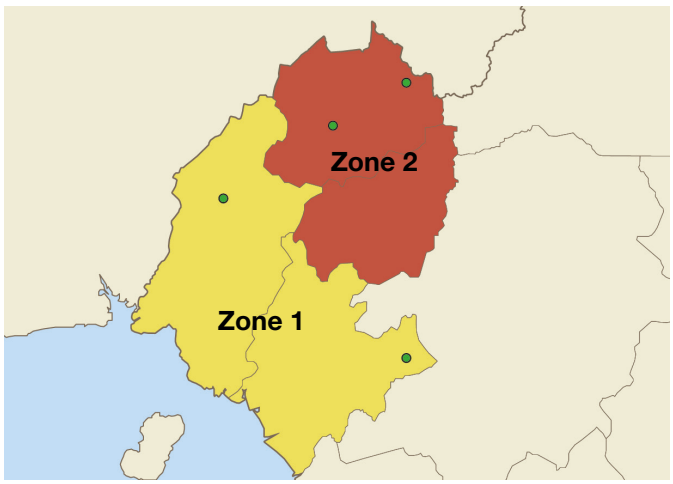


Figure 3.2

Random points (green) selected in the two identified ecological zones in southwestern Cameroon



3.2 Indicators

The use of ecological information outlined in this section of the protocol is designed to:

- strengthen the selection of sampling sites during the study based on previous experience and knowledge of how the environment can influence YFV activity;
- improve the understanding of the field studies results in terms of the external influences on the transmission of the YFV between and among the human, non-human primate and mosquito populations.

The ecological indicators that are considered to be most relevant to YF risk are priority indicators, which have the greatest influence on vector density and viral transmission, and secondary indicators, which are less influential but may also contribute to RA.

3.2.1 Priority indicators

Temperature

The collection and observation of temperature changes in the study area may increase the understanding of the relationship between climate, and YF transmission and outbreaks. Daily values of minimal, maximal and average temperature should be considered in the analyses. Temperature changes during seasonal and yearly timescales can be observed by obtaining time-series analyses at:

- low resolution for the area of study (satellite data for 10–30 years);
- high resolution, where possible, using data from meteorological stations located close to the sampling sites.

Rainfall

Seasonal and yearly rainfall changes and anomalies serve as a means to identify critical periods of vector development and increased risk of YF outbreaks. Rainfall data from satellites or in situ observations can illustrate frequency of wet days per month, intensity and duration of the rainy season, or daily or weekly rainfall. The best period to conduct a YF RA is at the end of the rainy season. In areas with two rainy periods, the assessment should happen at the end of the larger rainy season.

Vegetation coverage

Vegetation coverage is influenced by a series of factors (such as rainfall, temperature, humidity, urbanization and deforestation) and can vary on a seasonal basis. Vegetation has a direct influence on the density and size of the vector and non-human primate populations. Significant changes to vegetation coverage can lead to changes in the interactions between humans, non-human primates and vectors, and influence the transmission of the YFV. Satellite imagery, earth observations and vegetation indices are used to assess the recent changes in vegetation coverage and density.

3.2.2 Secondary indicators

Elevation

Elevation can represent specific environmental conditions that influence the presence, density and reproduction of vectors. Although an upper limit of 2300 metres above sea level is commonly accepted, regional and local differences may need to be further investigated.

Land use and industry

Human activities impact vegetation coverage largely by agriculture-related deforestation, and industry and urbanization. To support the RA, information related to industrial activities such as mining, oil exploitation, deforestation, may be obtained from government departments.

Population movement

In Africa, large migratory flows are observed due to reasons such as rural exodus, movements of religious groups (e.g. Mourides in Senegal), cross-border movements of seasonal workers and nomadic pastoral communities, trade routes (e.g. from the Sahel to the coast of the Gulf of Guinea), the phenomenon of new urban dwellers returning regularly to their rural communities of origin, and migration by populations fleeing armed conflicts.

In the Americas, the migration is related primarily to occupational activities. These human movements increase the risk of contamination of non-immune persons travelling in areas where contaminated vectors persist and, conversely, favour the introduction of the disease into previously silent YF areas.

In some cases, tourism activities can temporarily change the population in an area.

3.2.3 Additional indicators

In certain areas, the incidence of other mosquito-borne diseases, such as malaria, may be useful as a proxy indicator to provide further information on the appropriate conditions for the presence of vectors. The use of malaria as an indirect indicator is more relevant in Africa, where the peak of the responsible vectors for both YF and malaria occur primarily during the rainy season. Although large areas in several American countries are not endemic for malaria, they may face malaria vector density peaks towards the end of, or following, the rainy season.

3.3 Geographic information system and mapping tools[d]

The YF RA conducted in Cameroon in 2011 explored the availability of, and accessibility to, relevant ecological information and mapping tools. The ecological and environmental indicators aimed to support the planning of the RA and the interpretation of its results in south-west Cameroon. The tools and methodology may also be used to support future YF RAs planned for other YF-endemic countries.

The identification of the ecological zones in the area of interest should be done by integrating information on the climatic and vegetation conditions. This approach provides an understanding of the ecological zones that are most suitable for YFV transmission, due to their ability to support mosquito species and non-human primates capable of amplifying the virus.

Further research and a comprehensive literature search are required to determine the specific thresholds for each of the climatic variables considered to be relevant to YFV transmission, such as humidity, rainfall and temperature. When thresholds are not available, a notable variation may provide sufficient information (e.g. a sudden increase in rainfall). Once the thresholds have been identified, they should be integrated into the tool's code, and areas of low to high suitability should be represented in colour on the map. The tool will be useful in helping identify the distinct ecological zones within which the sites for the human, non-human primate and mosquito sampling will be randomly selected.

Once the serosurvey sampling is conducted in the sampling sites, high-resolution data and satellite images related to the ecological (climate and vegetation) and demographic (population density) conditions can be obtained to help strengthen the analysis and interpretation of the findings. This information can be downloaded through online tools and platforms as datasets, time series or images for use as layers in geographic information system mapping tools.

For future investigations and RAs, ecological and environmental information related to specific and predefined indicators (see Section 3.2) should be collected before and at the time of the sampling by the study team members, to support the use of remote-sensing satellite images, and time series of vegetation, population density and rainfall.

[d] *Available at http://iridl.ldeo.columbia.edu/home/.remic/.maproom/.Health/.Regional/.Africa*

4 Data handling, analysis and reporting

The data collected by the teams during the field mission need to be appropriately analysed and correlated to provide results, and to support vaccination plans and policies.

4.1 Data handling

All survey tools and databases to be used should be finalized before the field team is deployed. Upon obtaining the information, completed forms should be given to a database manager, who will enter the responses. All collections – whether it is a person, mosquito pool or non-human primate – should be coded in a standard way that will maintain anonymity, but will also allow the database manager to determine which field team collected the information at what location. The database manager should attempt to enter all of the data into the database and clean the data before the field team disperses, so that the team can ask questions about the information. Missing or incomplete data, and/or entry errors may occur, and the forms may need to be reviewed to ensure that only accurate data is used for the analysis. Adjustments must be made to avoid discrepancies.

Laboratory results will often be entered into a separate database. All tests results should be entered according to the corresponding code assigned to the person, mosquito pool or non-human primate samples. Furthermore, the database used by the laboratory should be compatible with the databases containing the survey information to allow these data to be combined for analysis.

4.2 Data analysis

The data should be analysed after the laboratory results arrive. Clearly established timelines of various testing results will improve the timeliness of the analysis and study completion.

As several institutions may be involved in the analysis of the results of the samples, it is important to ensure that the results and statistical treatment of the data is harmonized. The laboratory data should be analysed based on a match with surveyed individuals and their laboratory data.[25]

The main outcomes of interest in the analysis include:

Human samples

i. naturally acquired YFV antibodies
ii. susceptibility (i.e. people that are not protected and considered at risk for YFV)

Non-human primates samples

i. presence of competent non-human primates
ii. prevalence of YFV antibodies

Vectors samples

i. presence and type of competent mosquitoes
ii. density of vectors (e.g. the Breteau and Container indices).

The analysis includes considering influences from ecological factors. If changes in the YFV circulation are observed, ecological factors may provide some explanations on when and why these changes have occurred.

4.3 Reporting

At the end of the field mission, it is recommended to organize a debriefing and present the preliminary results of the RA. A calendar of future activities should be created, including the finalization of the laboratory results. A preliminary report should be submitted to the health authorities before the international experts leave the country.

A final technical report should be produced after all testing and analyses have been finalized. This document should also contain the conclusions of the field mission and the key recommendations from the experts of the YF group. The countries and other stakeholders should be informed of timelines for completion of the technical report to create appropriate expectations. (Depending on the timelines to complete testing, the final technical report can take several weeks to months to complete.) Furthermore, a final meeting to share the results, conclusions and recommendations should be considered.

5 Ethical considerations and protection of confidentiality[24]

5.1 Ethical considerations

5.1.1 Human serosurvey

Blood collections present a small risk to participants; the main complications are pain and a haematoma. Aseptic technique should be used; however, a very small risk of infection could remain. During the consent process, individuals should be advised to seek medical care if a complication occurs secondary to the venepuncture. If feasible, the field team will provide assistance by transporting the individuals to a health-care facility and advising local health-care staff of the situation.

Based on the data collected from all components of the assessment (human, non-human primates, vector and ecological factors), the World Health Organization's laboratory testing algorithm[e] should be used to establish the areas that are at increased risk for yellow fever (YF), and the Ministry of Health (MoH) – in conjunction with international partners – should decide whether or not to vaccinate certain populations.

The information gained from the RA will benefit all individuals in the country, and will help the MoH to determine YF vaccination strategies based on the potential risk in each ecologic zone. Additionally, individuals who were serosurveyed will benefit from participating because they receive health education on YF disease and prevention.

Field teams involved with serum collection should be given personal protective equipment (PPE) such as gloves.

5.1.2 Non-human primate serosurvey

Non-human primates will be purchased for sampling from a market or hunter according to local official regulations. It is important to consider the principles of animal protection and care, or similar regulations for animal manipulation and experimentation.

No non-human primates should be killed for the specific purpose of the study and endangered species are excluded according to the exclusion criteria.

Field teams involved with non-human primate sampling should be given PPE including, but not limited to, face mask, gloves and gowns.

5.1.3 Mosquito survey

The main ethical consideration for the mosquito survey is the human landing collection, which could indirectly expose the volunteers to mosquito bites. The ethical considerations of this method of collection are being debated. However, in absence of alternative method, it remains the only one technique available for sampling adult mosquito populations, particularly the sylvatic vectors of YF virus.

All team members and local personnel who are involved with the adult mosquito survey and collection should be trained, vaccinated against YF and given prophylactic antimalarial medicine according to national guidelines. In addition, informed oral consent is obtained from all team members and local personnel involved in the mosquito survey and collection (including volunteers involved in the human landing collection).

Due to the level of education and limited time for the investigation, the study protocol should be carefully explained to the legal representatives of each community to be visited to obtain their consent.

Informed oral consent is also obtained from the head of the households from which larval and adult mosquitoes are sampled.

5.2 Protection of confidentiality

Each study participant is assigned a study number and their corresponding blood sample should be labelled with only the study number. Information obtained about the participant – including YF vaccination status, age, sex and geographical location – should be related to the study number.

There should be no link between any participant and their samples to ensure patient confidentiality.

A password-protected study database should be compiled and maintained by investigators. All information obtained from the assessment should be kept in electronic files and spreadsheets on secure password-protected computers. All human data should be entered without specific identifying information.

[e] *See in Annex 7.*

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Annex 1 Yellow fever background

Basic facts[18]

Yellow fever (YF) is an acute infectious disease caused by YF virus (YFV), the prototypic flavivirus.

Overall, case fatality incidence is approximately 5%. However, among those with more severe disease, including jaundice, the incidence is as high as 20–50%.

YFV is endemic in tropical areas of **Africa** and **South America**.

There has been a dramatic increase in the disease incidence in the past 15 years.

There are three types of transmission cycles:[28]

- **Sylvatic (or jungle) YF.** In tropical rainforests, YFV circulates between non-human primates and tree-top canopy mosquitoes (i.e. *Haemagogus* spp. in South America and *Aedes africanus* in Africa). Acute infected monkeys transmit the virus to naive mosquitoes that feed on them. Those mosquitoes then acquire and carry the virus, and subsequently transmit the virus to other primates after an intrinsic incubation period. The infected mosquitoes bite humans entering the forest, resulting in occasional cases of YF. The majority of infections occur in young men working in the forest (e.g. logging workers).
- **Intermediate YF.** In humid or semihumid parts of Africa, small-scale epidemics occur in savannah areas located on edge of the forested area. Semidomestic *Aedes* spp. mosquitoes (that breed in the wild and around households) infect both non-human and human primates. Interaction between people and infected mosquitoes leads to transmission. This is believed to be the most common form of transmission in Africa.
- **Urban YF.** Large outbreaks can occur when infected people introduce the virus into densely populated areas with a high number of non-immune people. *A. aegypti* mosquitoes often breed around homes in containers and readily bite humans, making people a good urban vector for the disease. Infected mosquitoes transmit the virus from person to person.

Case definition and clinical features

Box A1.1 outlines the World Health Organization's case definition for YF.

Box A1.1 World Health Organization case definition for yellow fever surveillance[20]

Suspected case: Any person with acute onset of fever, with jaundice appearing within 14 days of onset of the first symptoms.

Probable case: A suspected case **and** one of the following:

- presence of YF immunoglobulin M (IgM) antibody in the absence of YF immunization within 30 days before onset of illness;
- positive postmortem liver histopathology;
- epidemiological link to a confirmed case or an outbreak.

Confirmed case: A probable case **and** one of the following:

- detection of YF-specific IgM;
- detection of a fourfold increase in YF IgM, or IgG antibody titres between acute and convalescent serum samples, or both;
- detection of YF-specific neutralizing antibodies

and

- absence of YF immunization within 30 days before the onset of illness;

or one of the following:

- detection of YFV genome in blood or other organs by PCR;
- detection of YF antigen in blood, liver or other organs by immunoassay;
- isolation of YFV

and

- absence of YF immunization within 14 days before the onset of illness.

Outbreak:[20] a single confirmed case of YF is sufficient to identify a potential outbreak, and justify planning for early investigation and intervention.

The majority of individuals infected with YFV are believed to be asymptomatic. In people who develop disease, the incubation period is typically 3–6 days. Initial symptoms often include:

- sudden onset of fever
- headache or backache
- muscle pain
- nausea
- vomiting
- red eyes (injected conjunctiva).

The incubation period is often referred to as the 'period of infection' and typically lasts 2–5 days). The symptoms are nonspecific and YF can be confused with other diseases that have similar presentations, particularly because jaundice may not be present in mild or less severe cases of YF. The less severe cases are often non-fatal.

Following the initial symptoms, a temporary 'period of remission' occurs.

After this brief remission, 15–25% of individuals who are infected will develop more severe symptoms, referred to as a 'period of intoxication'. Jaundice, haemorrhagic manifestations (bleeding from the gums, nose or in the stool, vomiting blood) and signs of organ (e.g. renal) failure may occur.

Diagnosis

Diagnosis is by laboratory testing for YFV-specific antibodies, YFV by isolation or YF viral RNA by real-time polymerase chain reaction. Two blood samples must be sent to a reference laboratory for confirmation.

Case management

Supportive treatment should be given; no specific treatment is available for YF.

In the toxic phase, supportive treatment includes therapies for dehydration and fever. In severe cases, death can occur 7–10 days after onset of the first symptoms.

See Table A1.1 for managing specific symptoms.

Table A1.1 Symptoms of yellow fever and their management

Symptom	Management
Fever	Paracetamol
Dehydration	Oral rehydration salts or intravenous fluids (depending on the severity of dehydration)
Restlessness	Diazepam
Malaria	Antimalarial (as recommended for the particular area)
Bacterial infection	Antibacterials (as recommended for the particular area)

Access to mosquitoes, including daytime-biting mosquitoes, should be prevented by keeping infected patients in areas with screens or by using a bednet. This is important to prevent the patient from infecting other people.

Prevention and control measures

Exposure to mosquitoes should be avoided by using products such as protective clothing and insect repellents.

Sleeping and living quarters should be screened.

An effective vaccine is available, and mass vaccination is a key intervention for outbreak control.

In urban areas, mosquito breeding sites should be destroyed.

Annex 2 Example checklist and briefing programme

Example checklist

Activity/Activité	Responsible/Responsable de l'activité Allocation/Imputation
MoH and WHO's authorizations/Autorisations du ministère et de l'OMS	MoH/WHO Ministère de la Santé/OMS
☐ Official request for technical support/Demande officielle d'assistance technique	MoH/Ministère de la Santé
☐ Official letter to health district authorities/Lettre du ministère informant les autorités du district de santé de la venue de la mission	MoH /WHO Ministère de la Santé/OMS
☐ WHO mission order/Ordre de mission OMS	WHO/OMS
☐ Ethics authorization/Autorisation éthique	MoH /WHO Ministère de la Santé/OMS
☐ MoH agreement letter/Lettre du ministère autorisant la mission	MoH /WHO Ministère de la Santé/OMS
Study site/Site d'étude	Distance/duration Distance/durée
☐	
☐	
Human resources/Ressources humaines	Origin/salaries or per diems Origine/salaires ou indemnités journalières
Means of transportation/Moyens de déplacement	
☐ Vehicles/Véhicules	
☐ Gazoline/Carburant	
☐ Tolls/Frais de péage	
Communication/Communication	
☐ Mobile modem and battery/Modem de téléphone portable et batterie	
☐ Phone cards/Cartes detéléphone	
☐ Global positioning system (GPS)/Système mondial de géolocalisation (GPS)	
☐ Satellite phone (Thuraya)/Téléphone satellitaire (Thuraya)	
Small materials/Petit matériel	
☐ Ladles/Louches	
☐ Larvae tubs/Bac à larves	
☐ White sheets/Draps blancs (en percale ou popeline) 4 metres × 4 metres	
☐ Lamps/Lampes torches	
☐ Round pilesfor torches/Piles rondes (alkaline) pour les torches	
☐ Spare bulbs for torches/Ampoules de rechange des torches	
☐ Ice coolers/Glacières	
☐ Larvae trays/Plateaux à larves	
☐ Insecticide Spray/Bombes insecticides	
☐ Bed nets/Tulle moustiquaire	
☐ Plastic glasses/Gobelet en plastiques	
☐ Cotton/Coton	
☐ Liquid nitrogen/Azote liquid	
☐ Markers/Marqueurs	
☐ Petri dishes/Boite de Pétris	
☐ Cages/Cages	
☐ Magnifying glass/Loupes	
☐ Entomological material/Matériel entomologique	
Office material/Matériel de bureau	
☐ Attendance sheets/Fiches d'émargement	
☐ Larvae prospecting forms/Formulaires de prospection larvaire	
☐ Prospecting summary sheets/Fiches récapitulatives de prospection	
☐ Mosquitos forms/Fiches de mise en lots des moustiques	
☐ Non-treated mosquitos forms/Fiches de mise en conservation des moustiques non traités	

Example programme for a pre-mission briefing with local personnel

The objectives of the pre-mission briefing with local personnel are to:

- instruct team members on the specific techniques used for the YF epidemiological and entomological risk assessment;
- adapt the standard methodological approaches and the key elements of the evaluation of the YF risk epidemic to the area of interest, with a focus on
  - conditions to implement/organize the field investigation
  - criteria that determine the choice of the sites to be investigated and appropriate environment to be prospected in these sites
  - serological sampling procedures for human and non-human primate populations
  - most appropriate sampling procedures for the collection of the YF vectors
  - treatment and storage of the collected vectors
  - methods to estimate vector indices (e.g. Breteau or Container indices).

Annex 3 African vertebrate hosts[27]

Species	Source	Comments	Location of origin
Cercocebus spp. (mangabeys)	(Rodhain, 1991) (WHO, 2004)	Forest dwellers; live in canopy, but can descend to ground level Viremia lasts 2 days Only *Cercocebus torquatus* develops a titre of virus high enough to infect mosquitoes	Africa
Cercocebus spp. (mangabeys)	(Mccrae and Kirya, 1982)	3 out of 3 individuals tested positive by haemagglutination inhibition and mouse protection neutralization test	Uganda
Cercocebus albigena (mangabey)	(Digouette et al., 1995)		East and central Africa, Uganda
Cercocebus albigena johnstoni (black mangabey)	(Haddow et al., 1951) (Taufflieb et al., 1971)	Exclusively arboreal Viremia lasted 2 days and viral titres were very low 22 out of 50 individuals immune to YF by intraperitoneal mouse protection test	Democratic Republic of the Congo, Uganda
Cercocebus torquatus (mangabey)	(Taufflieb et al., 1971)	Live in forests mostly at ground level Viremia lasts 3–4 days, with subsequent immunity	Africa
Cercopithecus spp.	(Findlay and MacCallum, 1937)	4 out of 10 individuals were seropositive to YF by intraperitoneal mouse protection test in a dilution of 1 in 8	Liberia
Cercopithecus aethiops	(Rodhain, 1991) (Digouette et al., 1995) (WHO, 2004)	Move from forest to plantations Regularly descends to ground level and venture in savannahs Intense viremia lasts for 3–4 days	East and central Africa
Cercopithecus aethiops (grivet monkey)	(Taylor et al., 1955)	23 out of 32 individuals had antibodies against YF by intracerebral mouse protection test Virus dose used: 100–200 minimum lethal dose	Sudan
Cercopithecus aethiops	(Monath et al., 1980)	2 out of 3 adults tested positive by haemagglutination inhibition and by complement fixation test No virus was isolated from blood, spleens or liver	Gambia
Cercopithecus aethiops (vervet monkey)	(Kirk and Haseeb, 1953)	2 out of 17 individuals were seropositive to YF Tested by intraperitoneal mouse protection test According to Kirk and Haseeb (1953), monkeys are unlikely to have played an important part in maintaining the existence of YFV in Nuba Mountains, Sudan, because their population was scarce	Anglo-Egyptian Sudan
Cercopithecus aethiops (grey vervet monkey)	(Kirya and Okia, 1977)	1 out of 3 monkeys had positive titre (1/40) to YFV by haemagglutination inhibition test	Uganda
Cercopithecus aethiops centralis Neumann	(Findlay and MacCallum, 1937)	1 out of 15 individuals was seropositive by intraperitoneal mouse protection test, titre 1/8	Sudan
Cercopithecus aethiops centralis	(Findlay et al., 1941)	Sera of 1 out of 14 monkeys had antibodies against YFV by mouse protection test	Sudan
Cercopithecus aethiops centralis	(Findlay and MacCallum, 1937)	1 out of 15 individuals had YF antibodies by intraperitoneal mouse test in a dilution of 1 in 8	Sudan
Cercopithecus aethiops centralis	(Haddow et al., 1951)	18 out of 63 immune individuals by intraperitoneal mouse protection test	Uganda
Cercopithecus aethiops centralis	(Lumsden and Buxton, 1951)	1 out of 6 tested positive by mouse protection test	Uganda
Cercopithecus aethiops centralis Neumann (grivet monkey)	(Findlay and MacCallum, 1937)	5 out of 20 were seropositive to YF by intraperitoneal mouse protection test in a dilution of 1 in 8	Uganda
Cercopithecus aethiops johnstoni	(Haddow, 1952)	2 out of 26 individuals were seropositive to YF, using protection tests	Kenya
Cercopithecus aethiops sabaeus	(Germain et al., 1981)	YF host	West and Central Africa
Cercopithecus ascanius	(Rodhain, 1991)	Live in canopy Intense viremia lasts 3–4 days	Africa
Cercopithecus ascanius	(Mathiot et al., 1990)	22 out of 29 were seropositive by hemagglutination inhibition (Clarke and Casals' method, 1958)	Central African Republic
Cercopithecus ascanius schmidti	(Taufflieb et al., 1971)	Live at the top of the canopy, and is therefore intensely exposed to mosquito bites	West Africa
Cercopithecus ascanius schmidti (redtail monkey)	(Haddow et al., 1951)	87 out of 162 individuals had YF antibodies by intraperitoneal mouse protection test	Uganda
Cercopithecus ascanius schmidti (Matshie) (redtail monkeys)	(Simpson et al., 1965)	6 individuals were shot for the study. Virus isolation was attempted by inoculating serum from each monkey into newborn mice. No virus was isolated. 5 out of 6 individuals had YF antibodies by complement fixation, haemagglutination inhibition and protection tests	Uganda
Cercopithecus ascanius schmidti (redtail monkey)	(Kirya and Okia, 1977)	Virus isolations from sera were negative. 10 out of 18 had antibodies against YFV by haemagglutination inhibition test. All had 1/40 titre of antibody, except one individual that had 1/20 titre	Uganda
Cercopithecus ascanius schmidti (redtail monkey)	(Haddow et al., 1951)	Mainly arboreal 1 out of 5 individuals was immune to YF by protection test	Democratic Republic of the Congo, Uganda
Cercopithecus ascanius schmidti (redtail monkey)	(Henderson et al., 1969)	2 out of 13 individuals were immune to YF	Uganda
Cercopithecus ascanius schmidti (redtail monkey)	(McCrae and Kirya, 1982)	23 out of 58 redtail monkeys had YF antibodies by haemagglutination inhibition test (titre 1:10) and 33 out of 58 tested positive by neutralization test. All the monkeys that tested positive by haemagglutination test were also positive by neutralization test	Uganda

Species	Source	Comments	Location of origin
Cercopithecus diana	(Taufflieb, et al., 1971) (Digouette et al. 1995)	Forest species Viremia lasts 3–4 days following experimental infection with YF, then development of specific immune bodies	West Africa
Cercopithecus diana diana	(Findlay and MacCallum, 1937)	1 out of 1 individuals was seropositive by intraperitoneal mouse protection test in a dilution of 1 in 8	Ghana
Cercopithecus lhoesti lhoesti	(Haddow et al, 1951)	To a large extent, terrestrial	Democratic Republic of the Congo, Uganda
Cercopithecus mona	(Mccrae and Kirya, 1982)	1 out of 1 individual was seropositive to YF by mouse protection neutralization test. However, haemagglutination inhibition gave a negative result	Uganda
Cercopithecus mona	(Rodhain, 1991) (Digouette et al., 1995)	Live in middle and lower parts of trees Intense viremia lasts 3–4 days, followed by formation of specific antibodies to YF	West Africa, Central African Republic
Cercopithecus mitis	(WHO, 2004) (Digouette et al., 1995)	Live in canopy	East and central Africa
Cercopithecus mitis doggetti	(Haddow et al., 1951)	Mainly arboreal	Democratic Republic of the Congo, Uganda
Cercopithecus mitis stuhlmanni	(Haddow et al., 1951) (Taufflieb et al., 1971)	Mainly arboreal; live in canopy 19 out of 34 individuals were immune to YF by intraperitoneal mouse protection test	Uganda
Cercopithecus mona denti	(Haddow et al., 1951)	Mainly arboreal	Democratic Republic of the Congo, Uganda
Cercopithecus neglectus	(Haddow et al., 1951)	Strictly arboreal	Democratic Republic of the Congo, Uganda
Cercopithecus nictitans	(Rodhain, 1991) (Digouette et al., 1995) (Taufflieb, et al., 1971)	Live in canopy, but can venture in savannahs and plantations Intense viremia lasts 3–4 days	East and central Africa
Colobus spp.	(Taufflieb et al., 1971)	The most arboreal species of African monkeys, very rarely go down to ground YFV was only isolated once from a monkey, a young *Colobus* sp.	Ethiopia
Colobus spp.	(Mccrae and Kirya, 1982)	8 out of 8 individuals were seropositive to YF by haemagglutination inhibition and mouse protection test	Uganda
Colobus spp. or *Procolobus badius waldroni*	(Findlay et al., 1936)	One individual found to be seropositive to YF by intraperitoneal mouse protection test used	Ghana
Colobus abyssinicus	(Digouette et al., 1995) (Rodhain, 1991)	Main species affected by YF Frequent positive serology to YF	East and central Africa
Colobus abyssinicus	(Andral et al., 1968)	8 out of 30 were seropositive by hemagglutination inhibition in 1962 In 1964, 5 out of 23 were seropositive to YF	Ethiopia
Colobus abyssinicus ituricus	(Haddow et al., 1951)	Strictly arboreal 110 out of 207 individuals were immune to YF, tested by intraperitoneal mouse protection test	Democratic Republic of the Congo, Uganda
Colobus abyssinicus ituricus	(Lumsden and Buxton, 1951)	7 out of 14 individuals had YF antibodies by mouse protection test	Uganda
Colobus angolensis adolfi-friederici	(Haddow et al., 1951)	Strictly arboreal	Democratic Republic of the Congo, Uganda
Colobus badius	(Rodhain, 1991) (Digouette et al., 1995)	Frequent positive serology to YF	West Africa
Colobus badius	(Monath et al., 1980)	9 out of 11 monkeys tested positive by haemagglutination inhibition and only 1 of them tested positive by complement fixation test No virus was isolated from blood, spleens or liver	Gambia
Colobus badius badius (Procolobus badius badius)	(Findlay and MacCallum, 1937)	3 out of 6 were seropositive by intraperitoneal mouse protection test in a dilution of 1 in 8	Ghana
Colobus badius ellioti	(Haddow et al., 1951)	Strictly arboreal	Democratic Republic of the Congo, Uganda
Colobus badius tephrosceles	(Haddow et al., 1951)	Strictly arboreal 4 out of 13 immune individuals by intraperitoneal mouse protection test	Democratic Republic of the Congo, Uganda
Colobus badius waldroni (Procolobus badius waldroni)	(Findlay and MacCallum, 1937)	1 out of 3 was seropositive byintraperitoneal mouse protection test in a dilution of 1 in 8	Ghana
Colobus guereza kikuyuensis	(Haddow, 1952)	2 out of 54 individuals seropositive to YF by mouse protection test	Kenya
Colobus polykomos	(Rodhain, 1991) (Digouette et al., 1995)	Frequently seropositive to YF	West Africa
Colobus vellerosus (Colobus polykomosvellerosus)	(Findlay and MacCallum, 1937)	3 out of 5 were seropositive by intraperitoneal mouse protection test in a dilution of 1 in 8	Ghana
Colobus vellerosus (Colobus polykomos vellerosus)	(Findlay and MacCallum, 1937)	1 out of 1 individual was seropositive to YF. Serum tested with intraperitoneal mouse test in a dilution of 1 in 8	Sierra Leone
Colobus verus	(Rodhain, 1991)	Frequent positive serology to YFV	West Africa
Erythrocebus patas (patas monkey)	(Taufflieb et al., 1971)	Live mostly on ground level, very rarely climbs into trees. Feed on plantations frequently. When infected with YF experimentally, viremia lasted 3–4 days, followed by development of specific antibodies to YFV	West Africa
Erythrocebus patas (patas monkey)	(Mathiot et al., 1990)	2 out 32 individuals were seropositive by haemagglutination inhibition (Clarke and Casals' method, 1958)	Central African Republic
Erythrocebus patas pyrrhonotus	(Haddow et al., 1951)	To a large extent terrestrial	Democratic Republic of the Congo, Uganda

Species	Source	Comments	Location of origin
Galago spp. (bush baby)	(Taufflieb et al., 1971) (Digouette et al., 1995)	Agile and fast moving animal, nocturnal and mainly arboreal. In East Africa, especially in Kenya, antibody rates were higher than in monkeys and it is possible that it was the main vertebrate host of the virus	East Africa
Galago crassicaudatus (bush baby)	(Taufflieb et al., 1971) (Rodhain, 1991)	Live in open forests and wooded savannahs but not in rain forests. Its distribution does not expand north of the equator. Exclusively nocturnal and therefore less exposed to mosquito bites than monkeys, who sleep at night The most susceptible African non-human primate to YFV. 50% mortality rate following experimental inoculation with YFV. Viremia lasts 4–8 days and antibodies appearing on day 10 following inoculation	East and central Africa
Galago crassicaudatus (bush baby)	(Haddow, 1952)	14/102 (13.7%) of bush babies were seropositive to YF in comparison to just 3/113 (2.6%) monkeys *Galago* spp. were possibly the main mammalian hosts of YFV in Kenya and the driest part of Uganda	Kenya, Uganda
Galago crassicaudatus lasiotis (bush baby)	(Haddow, 1952)	12 out of 60 individuals were seropositive to YF by mouse protection test	Kenya
Galago crassicaudatus panganiensis (bush baby)	(Haddow, 1952)	1 out of 5 individuals was seropositive to YF by mouse protection test	Kenya
Galago senegalensis (bush baby)	(Taufflieb et al., 1971)	The most widespread *Galago* sp. in Africa (west, east and central) Live in open forests and woody savannahs but not in rain forests. In some African countries like Malawi and Tanzania, serological surveys found no immune individuals whereas in Uganda and Zimbabwe, about 10% of animals were seropositive to YF	West Africa
Galago senegalensis (bush baby)	(Digouette et al., 1995)	Experimentally, viremia lasted 3–7 days followed by presence of specific antibodies. Also developed immunity without viremia (Bugher, 1951; Haddow, 1953) In West Africa, no evidence of their participation in circulation of YFV. Haddow and Ellice (1964) thought that YFV could be transmitted from *Galago* spp. to *Galago* spp. by ectoparasites, but this was never proved	West Africa
Galago senegalensis (bush baby)	(Bugher, 1951)	Potential host for YFV because multiplication and circulation of YFV in the blood, followed by immunity Viral titre can be elevated Little or no illness	Democratic Republic of the Congo
Galago senegalensis (bush baby)	(Taylor et al., 1955)	1 individual out of 56 was seropositive to YFV by mouse protection test	Sudan
Gorilla gorilla	(Digouette et al., 1995) (Bugher, 1951)	Live in restricted area and not common enough to be an important host	West Africa
Macaca mulatta (Indian rhesus monkey)	(Kirya, 1977)	3 out of 4 sentinel monkeys died from YF. YFV was isolated from the brain of one individual, and from the brain, blood and liver of the second individual	Uganda
Macaca sylvanus	(Taufflieb et al., 1971)	Only found in north Africa, where YF is not endemic Highly susceptible to YFV, like *Macaca mulatta*	North Africa
Pan troglodytes (chimpanzee)	(Digouette et al., 1995) (Haddow et al., 1951)	To a large extent, terrestrial Not a common species; therefore, cannot have important role in epidemiology of YF	East, west and central Africa
Pan troglodytes (chimpanzee)	(Osterrieth et al., 1961)	54 out of 94 monkeys had antibodies against YFV by mouse protection test	Democratic Republic the of Congo
Pan troglodytes (chimpanzee)	(Findlay et al., 1936)	1 out of 6 individual was seropositive to YF by intraperitoneal mouse protection test	French Guinea
Pan troglodytes verus (chimpanzee)	(Smithburn and Haddow, 1949)	Viremia lasted 3 days and antibodies against YF appeared on day 8	Central Africa
Papio spp. (baboon)	(Rodhain, 1991)	Live at ground level, sleep in trees Can move great distances and disseminate the virus	East, west and central Africa
Papio spp. (baboon)	(Taylor et al., 1955)	16 out of 20 individuals tested positive by intracerebral mouse protection test	Sudan
Papio spp. (baboon)	(Findlay et al., 1936)	1 out of 1 individual was seropositive to YF by intraperitoneal mouse protection test	French Guinea
Papio anubis	(Rodhain, 1991) (Digouette et al., 1995)	Savannah dwellers Develop intense but short viremia	East, west and central Africa
Papio cynocephalus	(Mathiot et al., 1990)	10 out of 27 were seropositive to YF by haemagglutination inhibition	Central African Republic
Papio cynocephalus	(Andral et al., 1968)	2 out of 3 individuals were seropositive to YF by haemagglutination inhibition in 1962 In 1964, 0 out of 5 individuals were seropositive	Ethiopia
Papio doguera tessellatus	(Haddow et al., 1951)	To a large extent, terrestrial 6 out of 20 immune individuals by intraperitoneal mouse protection test	Uganda
Papio papio	(Digouette et al., 1995)	Savannah dweller	Central and west Africa
Perodicticus potto (potto)	(Smithburn and Haddow, 1949) (Digouette et al., 1995) (Taufflieb et al., 1971)	Nocturnal animal, strictly arboreal, confined to tropical rain forests. Moves very slowly, making it an easy prey for mosquitoes Following experimental inoculation, developed a viremia elevated enough to infect mosquitoes, lasting 4–8 days. Do not show any clinical signs following infection with YFV. Antibodies to YF occurred 10 days after infection Too rare to have an important epidemiologic role	East, West and Central Africa

YF, yellow fever; YFV, yellow fever virus

Annex 4 African vectors[13]

Species	Breeding/living	Biting habit	Comments
Mosquitoes			
Aedes (Stegomyia) *aegypti*			
a) domestic form (*aegypti*)	Breeds in artificial container (e.g. containers for water storage, old cans, tins, used tyres)	Antropophilic Bites inside & outside during day, especially late afternoon All year around, including dry season	Distribution correlates with human behaviour Major & often the only vector involved in human-to-human transmission
b) wild form (*formosus*)	Breeds in natural water collections (e.g. tree holes, rock holes, fruit shells, crab holes)	Zoophilic Found during the rainy season & the beginning of the dry season	Small role in YF transmission (life span is short, it has little contact with monkeys)
Aedes (Stegomyia) *africanus*	Found in forest areas from the rain forest to the dry savannahs	Primatophilic After dusk in the canopy, but may bite at any time during the day when a convenient host is introduced in its activity area (intrusion effect)	Main vector in the rain forest and as an important vector in forest galleries
Aedes (Stegomyia) *opok*	Known from savannah areas	Less primatophilic than *A. africanus*	Virus often isolated from *A. opok* in Central African Republic and Côte d'Ivoire: Considered an important vector
Aedes (Stegomyia) *neoafricanus*	Known only from forest galleries of eastern Senegal		Never very abundant, but can act as an effective local vector (infection rate is high)
Aedes (Stegomyia) *luteo-cephalus*	In savannah areas, extending to the Sahelian zone, common in forested areas Breeds in tree holes	Primatophilic Bites monkeys after dusk in the canopy of forest/mangrove galleries	Incriminated in 1969 during the Jos plateau epidemic in Nigeria Main vector in savannah areas in west Africa
Aedes (Stegomyia) *simpsoni* group			At least three species isolated in Uganda in 1942 and many times during the Ethiopian epidemic 1960–62 In west Africa human-biting species of the *A. simpsoni* group were recorded only in Nigeria
a) *A. simpsoni*			Found in South Africa only
b) *A. lilii*		Non-primatophilic	Found in East Africa Not considered a YF vector
c) *A. bromeliae*	Vegetal breeding places (leaf axils of banana trees), tree holes	Day biter	Most probably the vector incriminated by Mahaffy or Haddow under name *A. simpsoni*
Aedes (Diceromyia) *furcifer-taylori* group	Savannah dwellers	Takes two blood meals during a single gonotrophic cycle	Species of this group first incriminated during the Nuba Mountains epidemic in Sudan in 1940 58 YFV strains were isolated from male and female wild-caught mosquitoes during an epizootic outbreak in Senegal in 1977
a) *A. taylori*	In the canopy of forest galleries	More simiophilic than anthropophilic	Main vector from monkey to monkey in the canopy of forest galleries
b) *A. furcifer*	In open savannahs and in villages	Anthropophilic Bites indoors and outdoors	Monkey-to-monkey transmission, but also human infections Main vector in intermediate epidemics: Gambia in 1978, Burkina-Faso in 1969 and 1983, Mali in 1987
Other mosquitoes		Have no strict trophic preferences, or do not bite primates	Never play an important role in dissemination or amplification of the virus
a) *A. metallicus*			
b) *A. vittatus*			
c) *A. dentatus*			
d) *A. stokesi*			
e) *Eretmapodites* spp.			
f) *Mansonia* spp.			Never play an important role in dissemination or amplification of the virus, because the virus's incubation period is too long
g) *Culex* spp.			
Ticks			
Amblyomma veriegatum	In nature of Central African Republic		YFV was twice isolated from ticks (once from males and once from eggs) More important role in maintenance of the virus, since the virus can be vertically transmitted

YF, yellow fever; YFV, yellow fever virus

Annex 5 South American vertebrate hosts[27]

Species	Source	Comments	Location of origin
Alouatta spp. (howler monkey)	(Digouette et al., 1995) (Rodhain, 1991) (Bicca-Marques and Santos de Freitas, 2010)	Very susceptible, usually infection is fatal Cannot be considered reservoir of YFV because of their high sensitivity to the disease. If they do not die from YF, they develop permanent immunity. Consequently, howler monkeys, like other monkeys and humans, only act as virus amplifiers	South America
Alouatta spp.	(Rifakis et al., 2006)	More than 100 individuals died from YF (retrospective study, no information regarding test used)	Venezuela
Alouatta caraya (black howler monkey)	(Vaconcelos et al., 2001)	9 out of 14 individuals killed for the study had YF antigens in liver samples detected by immunohistochemistry	Peru
Alouatta seniculus (red howler monkey)	(De Thoisy et al., 2001)	34 out of 97 monkeys were seropositive to YF by haemagglutination inhibition. Attempt to isolate YFV was negative	French Guiana
Alouatta seniculus (red howler monkey)	(de Thoisy et al., 2004)	18 out of 98 individuals had neutralizing antibodies to YF by haemagglutination inhibition. No clinical signs were observed. Positive titres (>1/40) were confirmed by seroneutralization to avoid cross-reactions, which consisted of mixing equal volumes of diluted serum with a virus suspension containing 100 TCID50. The mixture was inoculated onto monolayers of Vero E6 cell line and incubated for 5–7 days	French Guiana
Alouatta seniculus (red howler monkey)	(Downs, 1982)	YFV kills a high proportion of *Alouatta* spp. infected. A serological study revealed that the majority of the surviving monkeys were immune to YF	Trinidad
Aotus spp. (night monkey)	(Rodhain, 1991)	Very susceptible, usually fatal infection	
Aotus zonalis (night monkey)	(De Rodaniche, 1952)	1 out of 35 monkeys were seropositive to YF by intraperitoneal mouse protection test	Panama
Ateles spp. (spider monkey)	(Digouette et al., 1995) (Rodhain, 1991)	Very susceptible to YF, usually fatal infection	South America
Ateles fusciceps robustus (black spider monkeys)	(De Rodaniche, 1952)	2 out of 21 monkeys seropositive to YF by intraperitoneal mouse protection test No indications regarding titres used	Panama
Ateles geoffroyi panamensis (red spider monkey) and *Ateles robustus* (black spider monkey)	(De Rodaniche, 1957)	9 out of 100 individuals tested positive with intracerebral mouse protection test	Panama
Ateles geoffroyi panamensis (red spider monkeys)	(De Rodaniche, 1952)	4 out of 24 animals seropositive by intraperitoneal mouse protection test	Panama
Brachyteles spp.	(Brasil, Ministério da Saúde, 2005)	Susceptible to YF	Brasil
Callithrix spp. (marmoset)	(Digouette et al., 1995) (Rodhain, 1991)	Very susceptible to YFV; infection is usually fatal	South America
Cebus spp. (capuchin monkey)	(Digouette et al., 1995) (Strode, 1951) (Rodhain, 1991)	Naturally infected but lower mortality rate compared to other New World species. Usually develop fever but seldom die from infection	South America
Cebus apella (cebus monkey)	(Downs, 1982)	Usually do not die from infection and develop immunity Uncertain whether virus circulate in blood in titre high enough to infect mosquitoes	Trinidad
Hapale spp. (marmoset)	(Rodhain, 1991) (Soper, 1935)	Very susceptible, infection is usually fatal	South America
Lagothrix spp. (woolly monkey)	(Rodhain, 1991)	Low mortality rate following YF infection	South America
Marikina geoffroyi (squirrel marmoset)	(De Rodaniche, 1952)	5 out of 85 monkeys seropositive to YF by intraperitoneal mouse protection test	Panama
Marikina geoffroyi (squirrel marmoset)	(De Rodaniche, 1957)	12 out of 197 individuals had antibodies to YFV by intracerbral mouse protection test. An inoculum of 50 LD50 of French neuro-adapted virus per mouse was used. Serums giving positive results with 50 LD50 of virus were titrated against higher serial concentrations of virus. A high titre of antibody against YF suggested the specificity of the test and the unlikeliness of cross-reactions	Panama
Pithecia pithecia (white-faced saki)	(de Thoisy et al., 2001)	3 out 6 individuals had antibodies to YFV by haemagglutination inhibition	French Guiana
Pithecia pithecia (white-faced saki)	(de Thoisy et al., 2004)	2 out of 5 animals tested positive by haemagglutination inhibition and seroneutralization	French Guiana
Saguinus midas (tamarin)	(de Thoisy et al., 2001)	10 out of 38 seropositive animals to YFV by haemagglutination inhibition	French Guiana
Saguinus midas (tamarin)	(de Thoisy et al., 2004)	5 out of 42 animals had antibodies to YFV by haemagglutination inhibition. To avoid cross-reactions and misinterpretation, sera with titres ≥1:40 were subsequently confirmed by seroneutralization	French Guiana
Saimiri spp. (squirrel monkey)	(Digouette et al., 1995) (Rodhain, 1991)	Very susceptible to YFV	South America

TCID, tissue culture infecting dose; YF, yellow fever; YFV, yellow fever virus

Annex 6 South American vectors

Mosquito species	Breeding/living	Biting habits	Comments
Aedes (Stegomyia) *aegypti*	Breeds essentially in artificial containers (e.g. containers for water storage, discharged containers)	Anthropophilic Bites mostly inside houses and in the close vicinity all daytime Bites all year round, including during the dry season	Distribution coincides with human behaviour Only known natural vector involved in urban human-to-human transmission
Haemagogus (Haemagogus) *janthinomys*	Sylvatic; found essentially in rain forest Breeds in tree holes within the forest	Anthropofilic and simiophilic Bites mostly at the tree canopy but also attacks at the forest ground and in the nearby of gallery forest all day May bite all year round; much more frequently during the rainy season	Major vector involved in the non-human-to-human transmission across the American continent Due to its acrodenphilic habits, it is probably one of the main vector involved in the monkey epizooties in the rainforest and gallery forests surrounded by savannah
Haemagogus (Haemagogus) *albomaculatus*	Breeds in tree holes Restricted to northern regions of South America	Bites mainly inside and in the forest fringe, but may attack close or even inside hoses settled in the close vicinity of forests Day biter Primatophilic Bites frequently during the rainy season	Local vector (possibly the main vector in some areas of north-east South America)
Haemagogus (Haemagogus) *capricornii*	Breeds in tree holes Restricted to south and south-east regions	Day biter Mostly acrodendrophilic Primatophilic	Probably acts as secondary vector
Haemagogus (Haemagogus) *equinus*	Breeds in tree holes	Bites throughout the day, although with two peak periods	Probably secondary vector in northern South America and Caribbean regions
Haemagogus (Haemagogus) *spegazzinii*	Breeds in tree holes and bamboo Widespread in South America east Andes	Day biter	Probably acts as secondary/local vector
Haemagogus (Conopostegus) *leucocelaenus*	Breeds in tree holes Widespread in South America, and Trinidad and Tobago Sylvatic mosquito that flies long distances between patches of forests and gallery forests	Day biter Attacks inside and outside the rainforest and gallery forests in the savannah area Bites at the tree canopy, but is very frequent on the ground during the rainy season	Probably the major vector in tropical and subtropical southern cone (such as south and south-east Brazil and Argentina) Found naturally infected in Colombia, Bolivia, Argentina and Brazil
Sabethes (Sabethoides) *chloropterus*	Breeds in tree holes Widespread in Central and South America	Day biter Essentially restricted to the jungle and gallery forests Acrodendrophilic in the jungle, but may bite humans walking on the forest ground and in the nearby Persists through the dry season, and probably is a key to maintain the transmission in the unfavorable periods Biting frequency increases at the onset of the rainy season and peaks in the middle of the rainy season	Major vector involved in the monkey-to-monkey transmission across the American continent Probably the one of the most important vector involved in the monkey-to-human transmission in the natural environment High survival rates, with long gonothrophic cycles in the forest
Sabethes (Sabethoides) *glaucodaemon*	Breeds in tree holes Restricted to Brazil, Bolivia, Guyana and Suriname	Day biter Primatophilic Essentially sylvatic	Secondary/local vector
Sabethes (Sabethes) *cyaneus*	Breeds in tree holes Widespread in Central and South America	Day biter Canopy feeder	Secondary/local vector
Sabethes (Peytonulus) *soperi*	Breeds in tree holes Apparently restricted to Brazil, Argentina and Bolivia	Day biter Attacks mostly in gallery forests in the savannah areas Primatophilic	Secondary/local vector
Aedes (Ochlerotatus) *scapularis* *Aedes* (Ochlerotatus) *serratus* *Psorophora* (Janthinosoma) *ferox*	Essentially breeds in temporary pools on the forest ground, or in the nearby tree-shaded natural or modified areas	Opportunistic species in terms of trophic propensity; bites humans aggressively Bites mainly during the day, with peaks of frequency in the twilights (or in the early night)	Found naturally infected, but are not considered primary/effective vector
Aedes (Stegomyia) *albopictus*	Breeds in artificial and natural larval habitats (e.g. bamboo, tree holes) More common in the suburban areas with considerable vegetation coverage; may occur in the forest fringe	Opportunistic with considerable anthropophilic propensity Bites mostly outside houses, especially in areas with substantial vegetation coverage in villages Bites all year round, with a low density of mosquitoes in the dry season	Potential vector, as several populations from the Americas are noticeably orally susceptible to YFV in the laboratory Vertical transmission of the virus were experimentally confirmed Potential link between the sylvatic and rural/urban cycle, as it displaces between the semisylvatic and the modified environments

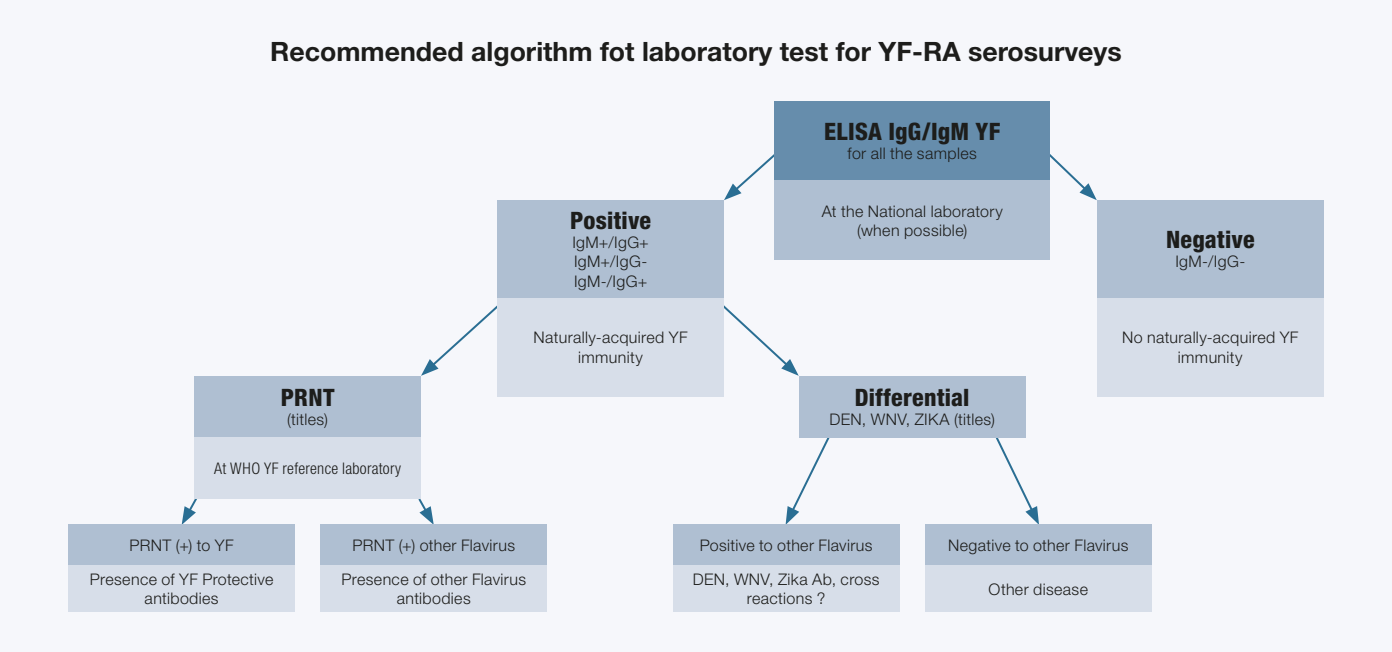
Annex 7 Testing algorithm for human and non-human primates samples

Flaviviruses tested

For serological testing, immunoglobulin M (IgM) and immunoglobulin G (IgG) are used as a screening test and quantitative plaque-reduction neutralization test is used as a confirmatory test when appropriate (see Figure A7.1 and Table A7.1).

For acute sample (samples collected within 10 days after the onset of disease), reverse-transcriptase PCR for detection of yellow fever RNA is also used.

Figure A7.1 Testing algorithm



In all scenarios immunoglobulin M (IgM) and immunoglobulin G (IgG) testing should be performed first; qualitative and quantitative PRNT are performed for any specific virus that is found positive by IgM and IgG test

Table A7.1 Testing results and possible interpretation

Test			Possible interpretation
IgM	IgG	PRNT test	
Acute specimen			
Neg	Pos	Neg	Previous flavivirus exposure with possible recent infection Convalescent specimen required
Pos	Neg	Neg	Possible recent infection, with cross-reacting flavivirus(es) not tested Convalescent specimen required
Pos	Neg	Pos	Recent primary infection with the flavivirus(es) found positive
Pos	Pos	Pos	Recent flavivirus(es) infection; primary or secondary
Convalescent specimen			
Pos	Neg	Neg	Possible recent infection, with cross-reacting flavivirus(es) not tested with delayed IgG (rare)
Neg	Pos	Neg	Previous exposure; no recent infection
Pos	Neg	Pos	Recent primary infection with the flavivirus(es) found positive with delayed IgG (rare)
Pos	Pos	Pos	Recent infection primary or secondary of the flavivirus(es) found positive by the test

IgG, immunoglobulin G; neg, negative; pos, positive; PRNT, plaque-reduction neutralization test

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