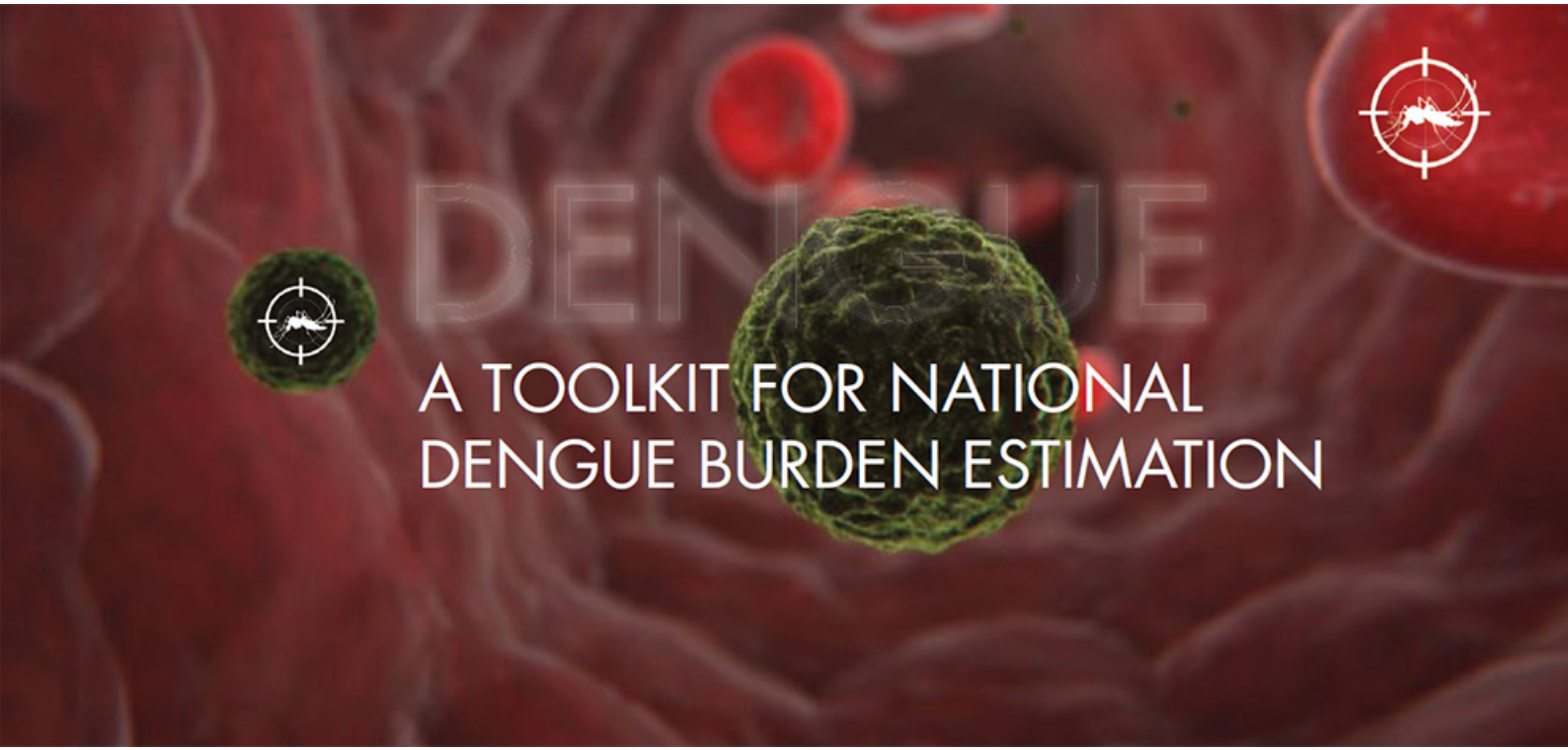

A TOOLKIT FOR NATIONAL DENGUE BURDEN ESTIMATION

World Health Organization

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CONTENTS

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ABBREVIATIONS

DENV	dengue virus
PPV	positive predictive value
UFI	unidentified febrile illness
DHS	Demographic and Health Surveys
IgM/G	immunoglobulin M or G
PCR	polymerase chain reaction
ELISA	enzyme-linked immunosorbent assay
HI	haemagglutination inhibition
PRNT	plaque reduction neutralization test
FOI	force of infection
WHO	World Health Organization

INTRODUCTION

Dengue is a viral disease vectored by *Aedes* mosquitoes that has spread throughout the tropical world since the mid twentieth century. Existing reactive control efforts have failed to stop the expansion of dengue virus transmission and many areas now have endemic circulation of all four dengue virus (DENV) serotypes. New strategies are needed to reverse this trend; and to be effective, they must be based on accurate quantitative information about the burden of dengue. The WHO *Global strategy for dengue prevention and control, 2012–2020* (*1*) highlights the urgent need to generate new estimates of the burden of dengue that are acceptable to all Member States.

While few dengue infections result in death, the high number of non-fatal cases imposes a heavy burden of morbidity and makes dengue an increasing priority in the more than 120 countries that it affects. Measuring the full burden imposed by dengue requires estimating the incidence of all levels of disease severity from minor to major. Severe cases can result in death or require lengthy hospitalization, with potential long-term side-effects; clinical dengue cases can overwhelm healthcare facilities; sub-clinical infections can limit attendance at work or school; and inapparent infections can act as a reservoir of infection that undermines surveillance and control efforts.

Understanding how these levels of burden are distributed over time, age and space in a particular country is important for:

- determining how to allocate optimally the limited resources available for dengue prevention and control;
- calculating the economic burden of dengue to build the case for investment in surveillance and control measures;
- in assisting policy-makers in allocating resources for sustained support to intervention programmes, based on impact;
- identifying gaps in surveillance or best practice clinical management;
- evaluating the impact of control activities locally and internationally;
- improving understanding of the local epidemiology and the potential for DENV spread;
- predicting the likely impact of new vaccination and vector control strategies, either alone or in combination;
- increasing recognition and treatment of potentially undiagnosed dengue cases in order to improve disease outcomes; and
- improving the targeting of vaccination programmes to areas where they will be most effective.

AIM

The aim of this toolkit is to guide countries on how to best estimate their current burden of dengue by combining existing data from dengue surveillance systems with ongoing research efforts to measure the community burden of dengue.

MEASURING BURDEN

LEVELS OF DISEASE SEVERITY OF DENGUE INFECTIONS

Human infection with dengue viruses can result in a broad range of disease manifestations of varying levels of severity (Fig. 1). These levels of infection severity are largely distinguished by how and where the patient is treated or managed. They include[1]:

1. Inapparent dengue

A dengue virus infection that does not result in any disruption to the daily routine of the individual. This includes infections that are entirely asymptomatic as well as those that have very mild symptoms that do not have an impact on the affected individual's routine but that may be detectable upon detailed examination or questioning.

2. Self-managed dengue

A dengue virus infection that results in disruption to the daily routine of the individual (e.g. missing school or work) but that does not result in seeking diagnosis or treatment from an official healthcare provider, i.e. a hospital or a clinic, and instead is self-managed or managed by friends or family possibly with non-prescription medication until recovery.

3. Non-severe dengue

The case classification of non-severe dengue is defined in the 2009 WHO guidelines (*2*) as travel to or resident in a dengue endemic area plus two of the following criteria: nausea or vomiting, rash, aches and pains, tourniquet test positive, leukopenia or any warning sign. This definition includes the classifications of dengue with and without warning signs and can include cases in whom dengue is either diagnosed using only clinical criteria (probable dengue in (*2*)) or those that are laboratory confirmed.

4. Severe dengue

The case classification of severe dengue is defined in the 2009 WHO guidelines (*2*) as patient presentation with severe plasma leakage, severe haemorrhage and severe organ impairment.

5. Fatal dengue

A death in which acute dengue virus infection is the sole or leading cause.

[1] While further subcategories of infection severity are also often recognized (e.g. hospitalized, non-hospitalized), for the purpose of burden estimation, the above categories make best use of the most commonly available data types.

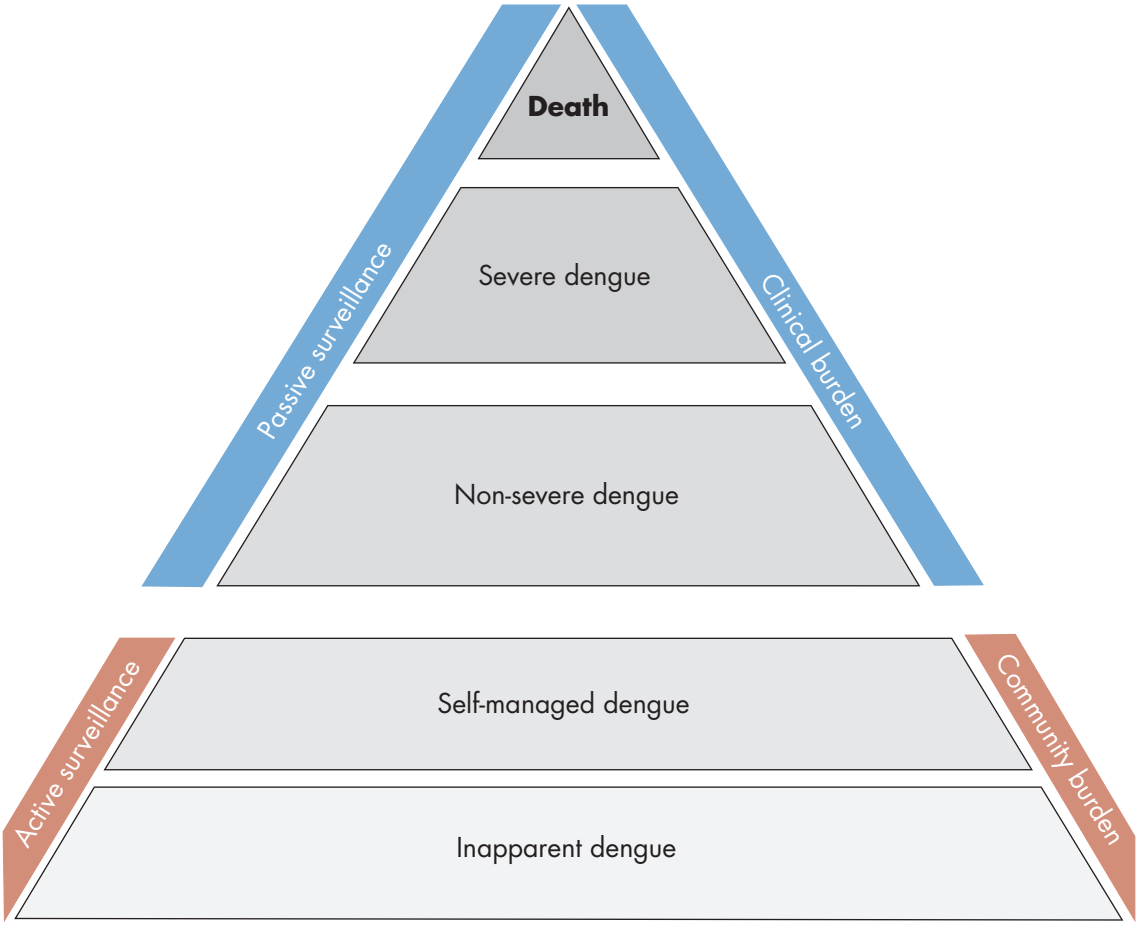


Fig. 1 Disease outcomes of a dengue virus infection divided by the clinical burden (blue line) that is measured by routine passive surveillance and the community burden (red line), which can only be measured by community-based sampling, i.e. enhanced disease detection, longitudinal cohorts and cluster studies.

More severe disease outcomes are more rare, but affected individuals are also more reliable to diagnose and more likely to seek treatment. Routine nationwide healthcare facility-based surveillance (hereafter routine surveillance) is the regular reporting of disease data by all institutions that see patients and are part of a reporting network (*3*). By design, these systems are only able to report cases that seek treatment (upper four levels in Fig. 1) and are typically less sensitive and specific as the severity of the disease becomes milder. To detect infections in a community that does not seek treatment (bottom two levels in Fig. 1) requires community-based sampling, where individuals are visited at home, school or their place of work and questioned about recent fever events and serologically tested for dengue virus infection. Such community-based surveys are often highly logistically and financially costly and are technically unfeasible at a national scale; as a result, they are only implemented at a few key sites.

The vast majority of routine surveillance data for dengue comes from passive surveillance, with some countries additionally having a small number of sentinel sites that conduct community-based sampling to measure the full spectrum of dengue disease severity in selected locations. This toolkit assesses why existing surveillance systems may not accurately represent the clinical burden of dengue, explains the methods for correcting inaccuracies and then advises on community-based surveys to measure the community burden of dengue.

WHY CURRENT SURVEILLANCE SYSTEMS MAY MISESTIMATE THE CLINICAL BURDEN OF DENGUE

Established national dengue surveillance systems provide the most comprehensive and frequently available source of data for estimating the burden of dengue. However, these passive surveillance systems will not capture all dengue infections, and the types of dengue infections they do capture are usually unrepresentative in severity, age distribution, geography and time.

First, there are epidemiological reasons why a dengue virus infection may not be detected. Because secondary infection with a different dengue virus serotype is more likely to lead to a more severe dengue disease outcome, younger individuals experiencing their first dengue virus infection are more likely to have an inapparent disease outcome and may not seek care or treatment for their mild illness. These age biases will vary in different areas that have a different history of dengue virus type invasion and persistence as well as different population demographics.

Second, there are sociodemographic factors that will affect whether an individual with a symptomatic dengue infection seeks treatment at an official healthcare facility (where they can be correctly diagnosed and treated) or chooses to self-treat (referred to here as treatment-seeking behaviour). Treatment-seeking behaviour can vary considerably depending on cost, accessibility and availability of care and, as a result, will differ in different areas and at different times (*4*).

Assuming that a symptomatic dengue infected individual seeks treatment at an official healthcare facility and thus has the opportunity to be recorded by routine passive sur-

veillance, there are further reasons why such individuals may be missed or incorrectly recorded. The rest of this section explains these common gaps in dengue surveillance and how they can be minimized so that surveillance data can be used to give a reliable measure of the clinical burden of dengue.

There are three main reasons why existing surveillance data may not give a good estimate of the true clinical dengue burden (see also the examples given in Table 1):

1. Incomplete coverage of the surveillance system
2. Cases detected are not representative of the total DENV infected population
3. Inconsistency of diagnosis, surveillance and treatment seeking in different places or over time

No surveillance system will have perfect coverage, representativeness and consistency, but that does not mean that its data cannot be useful for burden estimation. If current surveillance data have low, but known, coverage and good representativeness, reliable extrapolations can be made to areas where coverage is lacking (Fig. 2C). If a surveillance system has good coverage but low representativeness, it can be used to provide reliable estimates of subsections of dengue burden (e.g. cases but not deaths, Fig. 2B). Estimates of other subsections (e.g. deaths) can then be made using other data sources (e.g. data from sentinel sites, or from the published literature).

While most surveillance systems will be somewhere between these extreme examples, quantifying coverage, representativeness and consistency is essential to reliably estimate dengue burden and how it changes. The methods explained in the first section of this toolkit explain how these characteristics of the surveillance system can be practically measured.

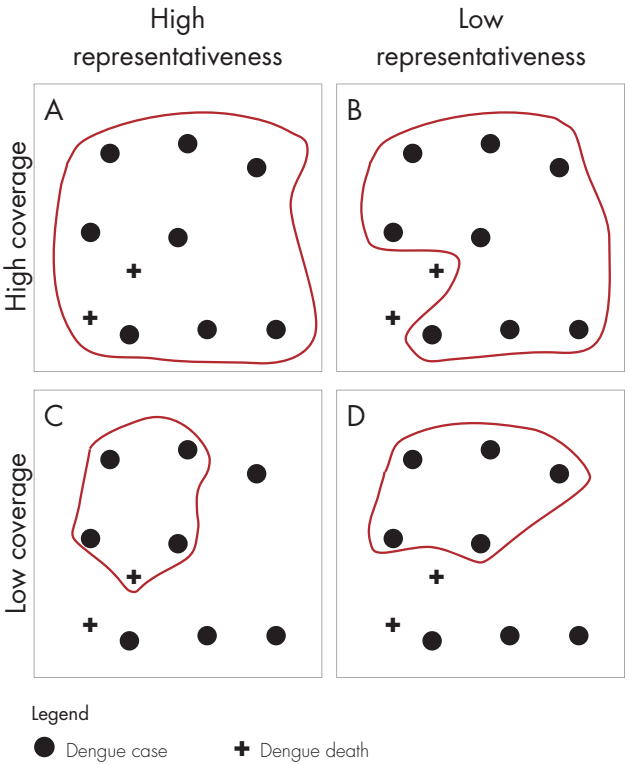


Fig. 2 The concept of surveillance system coverage and representativeness; surveillance system coverage (orange dotted lines) of dengue cases (filled circles) and deaths (crosses) can vary with respect to coverage and representativeness – changes in coverage and representativeness over time or space lead to changes in consistency.

Table 1. Understanding reasons why data from current dengue surveillance may under- or mischaracterize dengue burden

1. Coverage **Reasons why a surveillance system might miss dengue cases**	
1.1. Geography	• Isolated clinics are not part of the surveillance system
1.2. Policy	• Not legally notifiable • Private clinics are not part of the surveillance system
1.3 Capability and incentives	• Some facilities may not have sufficient laboratory support to accurately diagnose dengue • Some facilities may see reporting as a time burden that detracts from their time for patient care, or consider that the cases reflect unfavourably on their institution
1.3. Reporting fidelity	• Staff do not complete case notification
1.4. Misdiagnosis	• Dengue cases diagnosed as other febrile illnesses • Co-morbidities disguise dengue or lead to it not being reported as a contributing factor to disease
2. Representativeness **Reasons why a surveillance system might be more likely to miss some types of dengue infections more than others**	
2.1. Severity	• More severe cases are more likely to be correctly diagnosed • Reporting of deaths is subject to a higher degree of priority and independent review and thus deaths are correctly reported more frequently
2.2. Age	• Clinical dengue signs are more recognizable in children than adults • Sentinel surveillance may be confined to specific age groups, e.g. paediatric hospitals
3. Consistency **Reasons why surveillance systems may vary in coverage or representativeness**	
3.1. Time	• Time constraints during outbreaks mean staff do not have time to notify cases • Surveillance systems improve over longer time periods to increase the number of healthcare centres that are part of the system and provide greater capacity for laboratory diagnosis of dengue • Dengue clinical case definitions may change
3.2. Standardization	• Different physicians may or may not diagnose a dengue case or may classify it at a different level of severity based on differing personal professional opinion or prior experience.

TOOLKIT OVERVIEW

The aim of this toolkit is to estimate the national annual burden of dengue when applied in a given country or subnational area. This is achieved through a series of six sub-objectives that: assemble existing data (1.1), amend gaps in surveillance completeness (1.2), and correct for both over (1.3) and under (1.4) diagnosis to estimate the true clinical burden of dengue. These clinical burden estimates should then be combined with new or existing community-based surveys to estimate the symptomatic (1.5) and inapparent (1.6) community-based burden of dengue (Fig. 3).

The toolkit describes the use of simple data analysis techniques that can be implemented by programme managers in the health ministry and be paired with existing datasets. Even countries without extensive and well established surveillance systems are encouraged to at least complete the clinical burden estimation components (Table 2). However, most countries should be able to extend these efforts to estimate the full burden of dengue (clinical and community) as it brings many advantages for current and future control efforts (Table 2). This can be achieved by reviewing previously published research efforts to conduct fever cohorts and seroprevalence surveys or by partnering with researchers to conduct new surveys that fulfil the requirements of burden estimation set out in this toolkit.

Key parameter estimates from each of these separate studies can then be entered into a simple spreadsheet calculator to estimate the full burden of dengue at all levels of infection severity. Model-based estimates of key parameters will also be provided for comparison and for guidance when primary data are not yet available.

Countries are encouraged to document the burden estimation process in a formal, publicly available report. This report should include the sources for each piece of evidence used and key details about how it was collected and processed; each piece of evidence should conform to the Guidelines for accurate and transparent health estimates reporting, or GATHER, criteria (*5*). Technical support for implementation can be provided if requested.

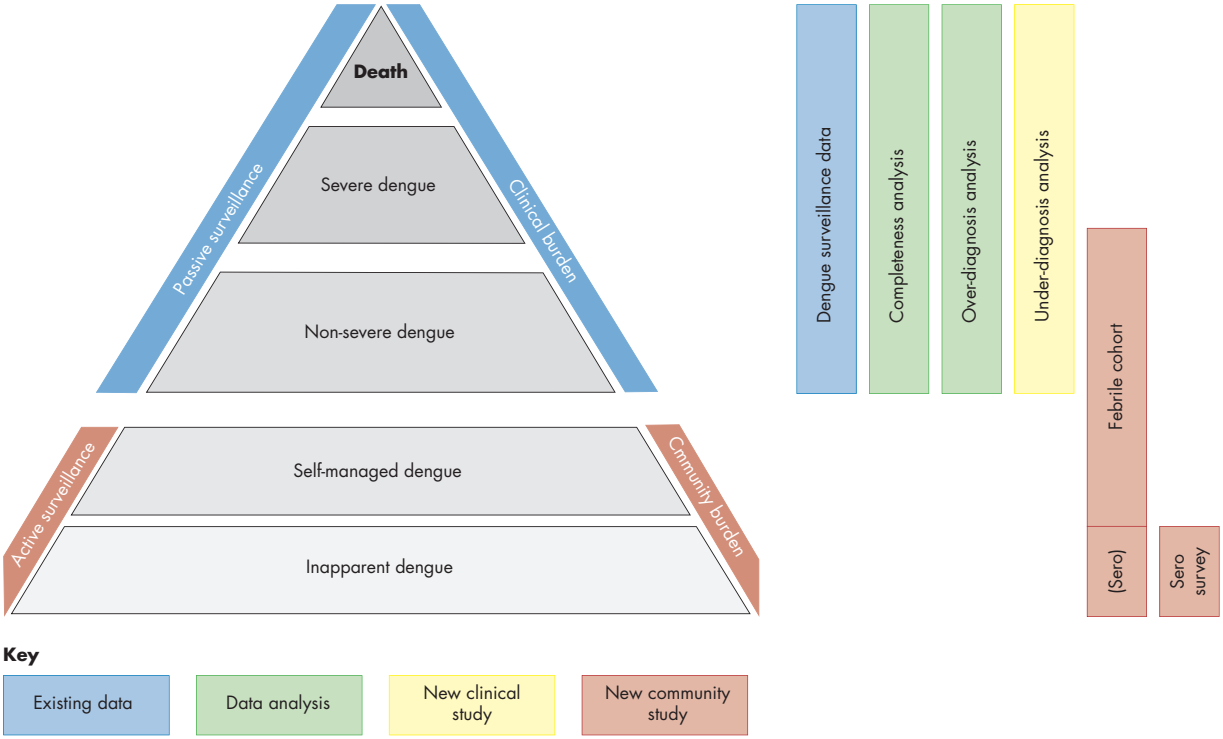


Fig. 3 Overview of data collection and analyses recommended in this toolkit and levels of dengue disease severity for which they provide burden estimates

Table 2. Feasibility, requirements and rationale for completing clinical or full burden estimation using the toolkit; the clinical burden of dengue can be estimated by completing activities (in orange), while calculating the full dengue burden requires completion of activities (in both orange and blue)

Dengue burden component	Analysis component	Data needed	Rationale for burden estimation	
			Clinical burden	Full burden
Clinical burden	Surveillance data	Existing data	• Gaps in surveillance • Gaps in best treatment practice • Direct medical costs	• Full economic burden • Optimize targeting of interventions • Evaluate effectiveness of control • Predict the effects of new interventions (e.g. vaccines) • Set achievable and measureable future goals
	Completeness analysis	Existing data		
	Under-diagnosis analysis	Existing data		
	Over-diagnosis analysis	Minor new study		
Community burden	Febrile cohorts	Existing published data / collaboration with research partners		
	Seroprevalence surveys	Existing published data / collaboration with research partners / in preparation for dengue vaccination		

SUGGESTED DENGUE SURVEILLANCE STANDARDS FOR BURDEN ESTIMATION

The methods suggested for estimating dengue burden in this toolkit are best suited to surveillance systems with the following criteria:

- Countries with regular dengue transmission with a national passive surveillance system established in all areas at risk.
- Countries in which at least a subset of dengue cases are confirmed using dengue-specific rapid or laboratory-based diagnostic tests (Table 8 (*2*)).
- Countries in which the number of dengue cases can be disaggregated by both disease severity (at least fatal/non-fatal) and age (at least children/adults).

The focus of this toolkit is to estimate the average burden of dengue for a given country and over a given year. Dengue burden and the way in which it is measured will vary considerably both sub-nationally and at different times of the year. It is therefore recommended that subsequent burden studies further investigate spatial and temporal variations in burden for surveillance so as to target control efforts appropriately (see 4.1–4). However, for the purposes of this first assessment we assume that dengue burden is being estimated at the national level for one given year.

1.1 COLLATING AND REPORTING DENGUE SURVEILLANCE DATA

As a first step, existing data on dengue cases from the past epidemiological year should be disaggregated by age, disease severity and method of confirmation, as shown in Table 3.

Disease severity is disaggregated into "dengue", "severe dengue" and "fatal dengue" following the case classification guidelines set out in the 2009 WHO criteria for dengue diagnosis (*2*). The dengue category includes cases both with and without warning signs. Fatal dengue, for the purpose of burden estimation, is defined as a death in which acute dengue infection was the sole or one of the primary causes. Cases should have mutually exclusive final outcomes, i.e. one infection results in dengue or severe dengue, not both.

It is recognized that there have been known issues around the practicality and utility of this revised case definition (*6, 7*), which have led many countries to adapt their own dengue case definitions or continue to use the 1997 WHO case definition (*8*). These inconsistencies complicate international comparison of dengue burden and, ideally, case definitions would be globally standardized. However, given that such standardization or reclassification of existing cases is outside the scope of this burden analysis, countries are encouraged to report cases as originally diagnosed and then to detail the case definitions used in the supplementary reporting questionnaire (Table 4).

Dengue cases at different levels of severity are to be disaggregated by patient age, and confirmation method. Notified cases refer to patients who have been reported as dengue through the routine notifiable diseases reporting system. Depending on how the surveillance system is designed, these cases may include some or all of the following: suspected dengue (where dengue is one of multiple suspected differential diagnoses), clinically confirmed dengue (where the case is confirmed on the basis of the patients symptoms) or test confirmed dengue (where

dengue has been virologically or serologically confirmed through testing of patient blood or serum). The criteria for national standard practice in notification should be included in the surveillance system questionnaire (**Table 4**).

In addition to notification, the disaggregation of the numbers of patient samples that were tested (either rapid test or laboratory-based) for suspected dengue infection and the numbers of these tested samples that had a positive result should also be recorded (**Table 3**). To enable international comparison of burden estimates, details of testing, including the criteria for who should be tested for dengue (e.g. everyone or just clinically complex cases), what tests are used to confirm dengue and the threshold used to define dengue positive from dengue negative (or indeterminate test result) should also be included in the surveillance system questionnaire (**Table 4**).

If recorded age groups are more aggregated in national dengue data than presented below (e.g. <15 years, 15+ years), then cases should be divided equally among all sub age bands.

Table 3. Data template for reformatting of national dengue surveillance data for clinical burden analysis

Age (in years)	Dengue			Severe dengue	Fatal dengue
	Notified (clinical and/or test confirmed)	Tested*	Test* confirmed	Notified (clinical and/or test confirmed)	Notified (clinical and/or test confirmed)
< 1					
1–4					
5–9					
10–14					
15–19					
20–29					
30–39					
40–49					
50–59					
60–69					
70+					

* Applicable tests include dengue-specific point of care (rapid) or laboratory-based tests that are appropriate for the day of illness on which samples are taken (see **Table 7**).

Table 4. Dengue surveillance system questionnaire to accompany national dengue surveillance data detailing each country's specific notification, clinical and laboratory case definitions

Notification criteria	
At what point in a patient's diagnostic history does it become compulsory for them to be notified to the national surveillance system ("notified dengue")? (tick all that apply)	Suspected dengue ☐ Clinically confirmed dengue ☐ Test* confirmed dengue ☐ Other (please specify):_____
Clinical case definitions**	
What **clinical** case definition is used for diagnosis of **dengue**?	
What **clinical** case definition is used for diagnosis of **severe dengue**?	
What criteria are used to determine if a death was due to dengue?	
Dengue testing	
What types of tests or assays are most frequently used to confirm dengue infection? And what percentage of samples are tested by each method? (e.g. NS1 rapid test, IgM/IgG ELISA, PCR)	
What are the threshold criteria for positivity for each laboratory-based assay? (e.g. copy number, PRNT50, titre)	
What are the criteria for when testing should be done? (circle one)	All suspected dengue cases Only sentinel sites Only clinically complex cases Only vulnerable individuals (e.g. pregnant) Other (please specify): _____

*Applicable tests include dengue-specific point of care (rapid) or laboratory-based tests that are appropriate for the day of illness on which samples are taken (see **Table 7**).

** Clinical diagnosis refers to dengue diagnosis based on patient symptoms and basic non dengue-specific laboratory procedures, e.g. full blood count or haematocrit.

1.2 COMPLETENESS ASSESSMENT OF NATIONAL DENGUE SURVEILLANCE DATA

The first step in burden estimation is to estimate the number of clinical dengue cases that have been diagnosed by physicians but not notified to the national surveillance system. This can occur due to a variety of factors. Table 5 summarizes the most important reasons of relevance to burden estimation.

Measures to reconcile each of these completeness gaps can be obtained through an internal assessment of the dengue surveillance system. Such assessments are a common feature of many dengue surveillance systems to ensure compliance with national notification practices (*3*).

Identifying healthcare facilities that are not part of the national notification system can be done by comparing the total number of healthcare centres that are registered and considered to be in an area at risk of dengue transmission with the number that have reported any dengue cases in the past five years. Outpatient only clinics should be excluded from this analysis if it is not routine practice to report dengue outpatients or if all dengue patients they do see are referred to centres that are able to report dengue. If outpatient clinics are included, it should be considered that they may have lower notification fidelity than other healthcare providers; and it should be ensured that calculations of notification fidelity include a representative number of outpatient clinics.

If private healthcare facilities are not required to notify dengue cases, the proportion of cases treated in the private sector can be estimated by comparing the total number of febrile illness episodes treated by major private healthcare providers with those in the public sector in equivalent catchment areas, e.g. cities or counties. Such a sample should ensure it includes a variety of socioeconomic levels (e.g. income levels) and service provision environments (e.g. rural vs urban). Alternatively, patients seeking treatment at public and private hospitals can be interviewed using a structured questionnaire that asks about their treatment seeking pathway. Such questionnaires are important for identifying individuals who might seek primary care and sometimes diagnosis in the public sector before choosing treatment in the private sector, or vice versa.

Notification fidelity can be estimated in a subsample of healthcare centres through retrospective analysis to count dengue cases in records of treatment (e.g. line lists of admitted patients or patient billing records) that can be compared with the total number of notified cases recorded in the dengue surveillance system over the same time period.

Table 5. Key reasons why dengue cases may be correctly diagnosed but not notified to the national surveillance system

Reason	Definition	Example
Gaps in surveillance system coverage	Logistical barriers to the notification of correctly diagnosed dengue patients	• Resource constraints mean small clinics lack the personnel or equipment (e.g. computer) to notify case • Inconsistent Internet access in remote areas prevent isolated clinics using a web-based reporting system • Only sentinel sites may record specific details about cases, such as disease severity
Private sector treatment	Dengue patients who are diagnosed in the private healthcare sector, which is not required to notify dengue cases	• Individuals with private healthcare insurance may choose to be treated in a private healthcare facility • An individual may choose to pay for private treatment to avoid long waiting times at public healthcare facilities during a dengue outbreak
Notification fidelity	Cases that have been correctly diagnosed as dengue but who are not notified or are incorrectly notified in the national surveillance system	• Insufficient training on how to notify leads to forms being incorrectly and inconsistently completed • Lack of resources during outbreaks mean staff prioritise patient treatment over completion of notification forms

Table 6. Dengue surveillance completeness questionnaire

Question	Answer
What percentage of all primary, secondary and tertiary healthcare centres are able to notify the following types of cases to the national surveillance system?	Dengue: __% Severe dengue: __%
Are private healthcare centres required to report all dengue cases to the national surveillance system? If NO, what percentage of all febrile illness cases are treated only in private healthcare facilities?	YES / NO __%
What is the notification fidelity* for the following types of cases?	Dengue: __% Severe dengue: __%

*Notification fidelity is defined as the percentage of diagnosed dengue cases in a healthcare facility that has access to the notification system that are correctly notified in the national surveillance system.

1.3 OVER-DIAGNOSIS ANALYSIS

As a result of imperfect diagnosis, other febrile illnesses (Box 1) may be misdiagnosed and notified as dengue (over-diagnosis). This is especially common in the mid to late phase of dengue outbreaks where dengue diagnosis is largely dependent on presumptive contextual evidence, especially when testing facilities are overwhelmed by excess requests (*9*). Misdiagnosis is also an issue in the context of outbreaks of other arboviral infections such as Zika virus disease and chikungunya which often share the same seasonal timing and non-specific clinical symptoms, while serological diagnostic tests for Zika virus disease and dengue can often cross-react (*10*).

The positive predictive value (PPV), or precision, of a dengue case definition is the proportion of true dengue cases among those that are notified as dengue (Box 2). A high PPV (close to 1) indicates low rates of over-diagnosis. PPV can be calculated using existing dengue rapid and laboratory-based testing data as long as they are representative of all clinically suspected cases. Some samples tested for dengue may be unrepresentative of all notified cases because testing is reserved for clinically complex cases, precautionary testing in vulnerable individuals (e.g. pregnant or the elderly) or may be underutilized in the later stages of dengue outbreaks due to laboratory capacity constraints.

Box 1

Other febrile illnesses that can be misdiagnosed as dengue

Depending on the context and phase of illness, dengue may commonly be misdiagnosed as:

- other arboviral infections (e.g. Zika virus disease, yellow fever, chikungunya)
- measles
- rubella
- adenovirus infections
- influenza
- typhoid fever
- malaria
- leptospirosis
- viral hepatitis
- Rickettsial infections
- bacterial sepsis

Source: reference (*11*).

Box 2

Calculation of sensitivity and positive predictive value of a dengue case definition

		Clinical diagnosis	
		Dengue	Not dengue
Diagnostic test	Dengue	A	B
	Not dengue	C	D

$$PPV = A/(A+C)$$

$$Sensitivity = A/(A+B)$$

To reduce these biases when calculating PPV, the cases included from the laboratory testing dataset should have the same age and weekly distribution as the notified cases dataset (i.e. all cases). This can be achieved by sampling selected cases from the laboratory dataset with their probability of being chosen dependent on the proportion of adult and children samples in each week in the notified cases dataset.

It must also be considered that dengue tests also have variable degrees of sensitivity and specificity (see Laboratory diagnosis and diagnostic tests (Chapter 4) of the WHO 2009 guidelines (*2*) and samples chosen for the analysis should be selected based on maximizing the performance of each test for the number of days between fever onset and sample collection (**Table 7**).

"True" dengue cases for the over-diagnosis analysis should be reserved for cases that satisfy the "highly suggestive" or "confirmed" definition in the WHO 2009 guidelines (*2*).

The PPV should be calculated separately for adults and children to reflect known differences in the specificity of dengue diagnosis (*12*); however, if dengue is primarily a paediatric disease in the area concerned only estimates for children should be made to make this study more feasible (**Table 9**).

Table 7. Time window for accurate diagnosis of dengue using different diagnostic tests

Method	Optimal time window for detection (days after onset of symptoms)
Viral isolation	1–5 days
Nucleic acid detection (PCR)	1–5 days
Antigen detection (NS1)	1–6 days
IgM ELISA or IgM rapid test	After 5 days
IgG (paired sera) by ELISA, HI or neutralization test	Acute sera 1–5 days; convalescent after 15 days

ELISA, enzyme-linked immunosorbent assay; HI, haemagglutination inhibition; Ig, immunoglobulin; PCR, polymerase chain reaction
Source: reference (*6*).

Table 8. Criteria for highly suggestive and confirmed dengue infections; note that IgM and IgG serological methods may cross-react with antibodies to other flaviviruses and therefore may be less accurate in regions where multiple flaviviruses co-circulate

Highly suggestive	Confirmed
One of the following: 1. IgM-positive in a single serum sample 2. IgG-positive in a single serum sample with an HI titre of 1280 or greater	One of the following: 1. PCR-positive 2. Virus culture-positive 3. IgM seroconversion (four-fold rise in titre) in paired sera 4. IgG seroconversion in paired sera or fourfold IgG titre increase in paired sera

HI, haemagglutination inhibition; Ig, immunoglobulin; PCR, polymerase chain reaction
Source: reference (*2*).

Table 9. Recording the results of the over-diagnosis analysis of notified dengue cases

Population (age in years)	Notified cases that tested positive dengue	Notified cases that tested negative for dengue	PPV of case definition (positive samples / total samples tested)
Children (< 19)			
Adults			

PPV, positive predictive value

1.4 UNDER-DIAGNOSIS ANALYSIS

While PPV of a case definition quantifies over-diagnosis and over notification of dengue, case definition sensitivity also needs to be known to quantify under-diagnosis of dengue. Dengue, particularly in its more milder disease forms, is frequently misdiagnosed as other febrile illnesses, especially in areas with high prevalence of other acute febrile illnesses (**Box 1**). Furthermore, many people with dengue infections do not develop highly specific clinical symptoms, and those that do seek care do not receive a final diagnosis – undifferentiated febrile illness, or UFI (*13–15*).

Despite their often mild disease outcomes, UFI dengue infections are an important part of the clinical burden of dengue because:

- they are improperly treated, typically with antibiotics or antimalarials resulting in a greater risk of anti-microbial resistance emergence; and
- they frequently overwhelm healthcare infrastructure during outbreaks which compromises care for other patients

To estimate under-diagnosis of notified clinical dengue requires new data collection under a prospective clinical study. This new study requires minimal new resources as samples can be collected and tested using the already established routine surveillance infrastructure and testing facilities.

The protocol for such a study should be based on the following major steps:

1. Selected healthcare facilities around the country should be chosen to take part at different times of the year (see Box 3 for area sampling strategies).
2. A clinical cohort of febrile illness patients should be enrolled at the point of first seeking care (e.g. emergency room, outpatient clinic, triage, etc.).
3. Exclusion criteria for enrolment should include negative for all other obvious causes of infection (e.g. existing chronic illnesses, other common febrile illnesses if relevant- malaria).
4. Samples (blood, serum or plasma depending on routine practice and diagnostic method used) should be obtained at the relevant disease time-point (see Table *7*) from all enrolled patients and sent for diagnostic testing for dengue using the routine testing protocols.
5. Clinical diagnosis of enrolled patients should be recorded with no modification to standard clinical practices.
6. Diagnostic test-negative patients can be discarded from the study.
7. Sensitivity is calculated as the proportion of true dengue infections that receive a clinical diagnosis of dengue (**Box 2**).
8. Under-diagnosis (as measured by case definition sensitivity) should be disaggregated by age of patients because these are known confounders in clinical diagnosis (*16*) (**Table 10**). However, if dengue is primarily a paediatric disease in the country in which burden estimation is being performed, case efforts should focus on maximizing sample size in paediatric populations.

Countries may have already undertaken research to estimate the sensitivity of their clinical case definitions for dengue. If this has already been performed, this data can alternatively be used to fill out the sections in **Table 10**, however it is important that such studies use the same denominator as suggested above, i.e. all febrile illness patients without obvious alternative cause not, for example, patients with dengue-like illness which may overestimate clinical case definition sensitivity.

Table 10. Recording the results of the under-diagnosis analysis

Population (age in years)	Test positive dengue cases in UFI sample	Test positive and clinically diagnosed dengue cases in UFI sample	Sensitivity of dengue case definition (clinical and test positive / test positive)
Children (< 19)			
Adults			

UFI, unidentified febrile illness

Box 3

Sampling strategy for new clinical and community-based surveys

For the purpose of this burden estimation exercise these new surveys are intended to generate estimates of under-diagnosis (1.4) and incidence (2.1 and 2.2) that are nationally and annually representative. Further studies can also be planned to estimate subnational or intra-annual variation in burden.

Where should these studies take place?

Ideally to be nationally representative, study sites (healthcare centres and their catchment populations) should be sampled with their probability of being chosen proportional to their catchment population (i.e. more sites in densely populated areas). However, given the need for collaboration between established research projects and existing routine surveillance activities, an element of convenience sampling is advised and integration of studies should take priority over strict geographically representative sampling. Countries should aim for representation from each major dengue-endemic administrative subregion (e.g. North, South, East, West, or provinces/states if feasible) and include at least some rural or peri-urban sites proportional to the percentage of the national population that lives in each of these areas.

When should these studies take place?

To be annually representative, sampling should occur across a 12-month period with sample number proportional to when most dengue infections occur (i.e. more samples in the dengue season). This can be achieved in clinical studies by sampling every 10th febrile illness patient (depending on sample number). However, because of under-diagnosis of dengue during the early phase of an outbreak and over-diagnosis of cases in the mid to late stages of an outbreak and to make studies more practical, this might change to every 5th case one month before the typical dengue season time, then transition to one every 20th case once the dengue outbreak* begins.

Who should be sampled?

For clinical studies, samples should reflect who seeks care, so there is no need to stratify by any particular criteria. Febrile illness and seroprevalence cohorts should ideally be representative of the resident population. However convenience sampling is often required to make such cohorts feasible and as a result school children or employees of a particularly company are typically chosen for follow-up. Community-based studies should aim for measuring the incidence of symptomatic and inapparent dengue infection in at least one adult and one child cohort.

Sample sizes?

Required sample size for both clinical and community-based studies will be highly dependent on sub-national variance in transmission intensity and diagnostic standardization making exact numbers difficult to generalise to different settings. Past community-based febrile illness cohorts and serological cohort studies have included around 1000 individuals followed for a period of at least one year. For the under-diagnosis analysis, countries should aim for between 1000–2000 samples with further samples required if highly variable sensitivity results are found.

Further details

For more details on sampling strategy and sample size calculation please consult WHO guidelines on dengue serosurveys for vaccine targeting (*17*).

* The definition of a dengue outbreak will vary from country-to-country but is typically when the weekly number of cases exceed two standard deviations of previous five non-outbreak year's equivalent weeks case numbers.

Box 4

Gold standard locations for burden estimation

Integration of studies

Each of the studies and data analyses recommended in these guidelines measures unique and non-overlapping segments of dengue's burden. Therefore it is important that different types of studies (1.1–4. and 2.1–2) occur in the same locations and ideally at the same times. This will generate "Gold standard" sentinel sites for burden estimation where the burden of dengue at all levels of severity from inapparent infection to death is known.

Maximising existing data

Community-based surveys are typically costly and usually only conducted in research settings as opposed to routine public health activities. However, many febrile illness cohorts and serological cohort studies have been previously carried out in a variety of settings. Countries are encouraged to integrate burden estimation activities with the past or on-going efforts to measure community burden. This could include research projects, but also preparatory or control-arm studies for vaccine or drug trials (*18*,*19*).

This will establish a network of gold standard burden estimation sites in the same location as previous cohort studies. Integration of clinical and community burden measurement activities has the potential to reduce the cost and improve the accuracy of burden estimation.

1.5 FEBRILE COHORTS

A high proportion of symptomatic dengue infections (estimated at around 70%; range 40–82%) (*17*) will not seek formal healthcare but will still develop disease symptoms. Estimating this subclinical symptomatic burden is important because affected individuals may still have chronic effects, e.g. prolonged joint pain, that may lead to significant productivity losses in a very large number of individuals. Many of these individuals may also seek informal healthcare and subsequently have worse or extended disease outcomes.

A self-managed dengue infection is defined as an infection of sufficient severity to disrupt the daily routine of the individual and will likely result in not attending work or school. As such events occur outside formal healthcare settings, a community-based fever survey is needed to measure their incidence.

FEBRILE ILLNESS SURVEYS

1. Selected schools (*20*) and workplaces (if dengue in adults is common in the affected country) (*21*) should be identified, and pupils and employees recruited into a fever cohort where each individual's age, gender and address are recorded (**Table 11**).
2. Individual school or work absenteeism should trigger a home visit to record the reason for absence.
3. If the absence is due to acute febrile illness, blood samples should be obtained from the individual on the day of the visit.
4. No attempts should be made to increase or decrease existing barriers to treatment for the infected individual, and any treatment seeking should be initiated by the individuals themselves and not directed by the study organizers.

5. The infected individual should be revisited (at home or school/workplace) at least 2 weeks after the first visit. During this visit the subject (or subject's parents in the case of schoolchildren) should complete some brief questions on:
   - Did they seek treatment for this acute illness episode?
   - If so, where did they seek treatment (e.g. public or private hospital, emergency department or outpatient clinic)?
   - If so, when did they seek treatment (e.g. 2 days after onset of illness)?
   - If so, what was your final diagnosis when you sought treatment (e.g. dengue or other non-dengue illness)?

   If IgM/IgG ELISA methods are being used to confirm infection, then convalescent samples should also be obtained from the individual during this visit. Additionally, if the study is done in an area with known circulation of other flaviviruses, then serological ELISA methods should be cross-validated using more sensitive methods (see (*22*) for details).
6. All samples should be tested to confirm or reject dengue infection (see **Table 8**).
7. The fever cohort study should run for a minimum time period of one calendar year. Additional individuals may need to be recruited into the cohort over time (ideally, at annual intervals) due to dropout.

Alternatively, fever cohorts can be household based with a similar design (20). In these household-based febrile cohorts, households are enrolled at baseline and, as opposed to absenteeism triggering follow-up visits, households are visited at fixed time intervals (e.g. every 6 months) and each participant completes a questionnaire to retrospectively collect data on fever and treatment seeking. These types of experimental designs work best when also taking periodic serological samples (see below) as this allows confirmation of reported dengue-like illness through monitoring an individual's seroconversion. These approaches may give more representative estimates of burden in adults as workplace-based cohorts may introduce selection bias for higher socioeconomic status than the general population.

Table 11. Output measures for reporting febrile illness cohort results

Measure (within cohort)	Number (child cohort)	Number (adult cohort)
Person-years of observation (number in cohort multiplied by time they were observed for)		
Confirmed apparent dengue infections		
Confirmed apparent dengue infections that sought treatment*		
Incidence of notified dengue cases in the area and during the time of the cohort study per 100,000 residents		

* Should only include treatment seeking to healthcare centres that are part of the national dengue surveillance system.

MEASURING INAPPARENT INCIDENCE WITHIN FEBRILE ILLNESS COHORTS

Inapparent incidence can also be measured within the same febrile illness cohorts with minimal additional sampling and testing commitments. Measuring the incidence of inapparent infection requires two additional steps to the above fever cohort:

1. At enrolment, a blood sample is taken from each individual and tested for prior dengue exposure with IgG ELISA.
2. Each subsequent year, every individual in the cohort is sampled and tested for IgG and IgM ELISA.

Further details on fever study implementation can be obtained from previous published febrile illness cohort studies (*20, 21*), particularly with regards to laboratory test quality control, informed consent, or participants and maximizing participation rate.

Many dengue febrile illness cohorts have already been conducted in a variety of areas worldwide, and stakeholder countries are encouraged to make use of existing and ongoing research activities to obtain this information (**Box 4**). Finally, it should be noted that other methods are available for measuring apparent dengue infection incidence that may give estimates of comparable accuracy. In particular, index household studies (*23*) where patients are recruited at the clinic level, then members of their immediate family and local neighbours are tested for recent dengue infection, may give comparable estimates of incidence, particularly in areas where a high proportion of individuals seek care. It should also be noted that in the absence of dengue-specific data on treatment seeking, treatment seeking rates for fever in children can be obtained from Demographic Health Surveys (DHS, www.dhsprogram.com) and national health surveys, which are frequently conducted in many countries and made publicly available.

Table 12. Serological fever cohort study additional output measures

Measure (within cohort)	Number (school-based cohort)	Number (adult-based cohort)
Total primary dengue infections in the cohort (IgG negative at enrolment then IgG positive at annual follow up)		
Total post-primary dengue infections in the cohort (IgG positive at enrolment then IgG and IgM positive at annual follow up or confirmed dengue following house visit triggered by school or workplace absenteeism)		
Total dengue infections (primary dengue infections plus post-primary dengue infections)		

1.6 MEASURING TOTAL INFECTIONS USING CROSS-SECTIONAL SEROPREVALENCE SURVEYS

Due to the high prevalence of inapparent dengue infections, the only reliable method for detecting the total number of dengue infections is through community-based serological surveys.

Measuring the total number of dengue infections (including asymptomatic infections) is important for predicting the impact of interventions such as vaccines and vector control that aim to prevent people from being infected. Determining how many infections need to be prevented to avert a clinical case or a case of severe dengue can be used to set goals and to evaluate new interventions or control strategies.

The number of total dengue infections can be measured through cohort-based longitudinal surveys where seroconversion is directly observed in paired samples from the same individual (section 1.5), or cross-sectional age-stratified seroprevalence surveys where accumulation of dengue antibody over time (age) is measured. Each of these methods has its advantages and disadvantages (summarized in Table 13).

While seroprevalence surveys have conventionally been considered a research-based activity, the growing recognition of the importance of the community burden of dengue to wider dengue control means there is a growing need to incorporate seroprevalence surveys into routine dengue surveillance.

Comprehensive guidelines on how to conduct age-stratified cross-sectional seroprevalence surveys are contained in *Informing dengue vaccination programs: best practices for conducting a serosurvey* (*22*), which includes advice on site choice, sample size and standard operating procedures. Guidance on serological cohort study design can be found in previously published sources such as in (*24*) and (*25*).

To be useful for burden estimation across all age ranges, the seroprevalence survey results must be used to calculate force of infection (FOI). FOI measures the annual rate at which susceptible individuals acquire infection and methods for its calculation can be found in section 4.1.4. in (*22*). The extracted FOI estimate can then be used to calculate infection burden using the burden calculator.

Table 13. Advantages and disadvantages of serological cohort studies and age-stratified seroprevalence surveys

Serological cohort studies	Seroprevalence surveys
Advantages • Incidence directly observed • More precise estimate of how incidence varies within the year • Can monitor serotype-specific incidence (if a subset are cross-validated with PRNT [plaque reduction neutralization test]) • Can be an addition to planned or existing febrile illness cohorts Disadvantages • Often limited to narrow age groups • Expensive, time-consuming and may require specialist equipment if results need to be serotype specific	Advantages • More suited for estimating long-term average incidence • Can measure (or predict) incidence across many age groups • Usually cheaper, quicker and more simple than cohort studies Disadvantages • Incidence indirectly observed (have to make assumptions of stable incidence and age-independent susceptibility) • Not serotype specific (unless done with PRNT, which limits sample size and feasibility)

BURDEN CALCULATION

2.1 USING THE BURDEN CALCULATOR*

The burden calculator is a spreadsheet-based tool that combines the information supplied in sections 1.1–6 to estimate national dengue burden. Each sheet in the burden calculator corresponds to a different type of data.

*Please contact WHO for the excel based burden calculator. Email: dengue@who.int

Raw data should be entered in the **green** coloured cells, while **orange** coloured cells indicate automatically calculated intermediate or final outputs.

While ideally data from all of the studies suggested in sections 1.1–6 should be entered, if some of this information is not available, approximations can be made from within the range of data from previous studies shown in **red** coloured cells. It is also important to represent uncertainty in some of these measurements. This is achieved in this burden calculator through the use of additional columns for multiple experiments or surveys.

Step 1: Enter age and severity stratified notified case data (see Table 3 and section 1.1) in Sheet 1 of the burden calculator.

	A	B	C	D	E	F	G
1							
2	1.1. Routine surveillance data						
3							
4	Age	Non-severe dengue			Severe dengue	Fatal dengue	
5		Clinical and/or test confirmed	Samples tested	Samples positive	Clinical and/or test confirmed	Clinical and/or test confirmed	
6	0-1	223	8	2	2	0	
7	1-4	3314	123	31	27	7	
8	5-9	5976	221	55	48	12	
9	10-14	5350	198	49	43	11	
10	15-19	4869	179	45	39	10	
11	20-29	12039	446	111	97	24	
12	30-39	7041	260	65	57	14	
13	40-49	4431	164	41	36	9	
14	50-59	2825	105	26	23	5	
15	60-69	717	27	7	6	2	
16	70+	717	27	7	6	1	
17	Total	47502	1758	439	384	95	
18							

Step 2: Enter completeness metrics (see Table 6 and section 1.2) in Sheet 2 of the burden calculator stratified by disease severity.

	A	B	C	D	E	F	G	H
1								
2	1.2. Completeness metrics							
3								
4	Question		Suggested range of values	Value (Experiment 1)	Value (Experiment 2)	Value (Experiment 3)	Value (Experiment 4)	Value (Experiment 5)
5	What percentage of all primary, secondary and tertiary healthcare centres are able to notify the following types of cases to the national surveillance system?	Non-severe dengue	50-100	100	100	0	0	0
6		Severe dengue	20-100	10.8	10.8	0	0	0
7	Are private healthcare centres required to report all dengue cases to the national surveillance system? If YES: leave blank, If NO, what percentage of all febrile illness cases are treated only in private hospitals?		10-50					
8	What is the percentage notification fidelity* for the following types of cases?	Non-severe dengue	80-100	80	90	0	0	0
9		Severe dengue	90-100	100	100	0	0	0
10	**Total completeness %: Non-severe dengue**			**80**	**90**	-	-	-
11	**Total completeness %: Severe dengue**			**10.8**	**10.8**	-	-	-
12								
13								
14								
15	* notification fidelity is defined as the percentage of diagnosed dengue cases in a healthcare facility that has access to the notification system that are correctly notified in the national surveillance system.							
16								

If private sector healthcare facilities are required to notify all dengue cases to the national surveillance system, leave row 7 blank.

If more than one completeness assessment has been performed or there are significant regional differences in completeness, it is advised to enter the separate results from separate surveys in different columns ("Experiment 1", "Experiment 2", etc.).

If data are unavailable for any section, reasonable values can be chosen based on previous surveys. These range of values are shown in the red boxes. If estimates from these red boxes are used, it is recommended that a broad range of values be used as opposed to a simple average to appropriately represent uncertainty in this estimate (e.g. enter 50, 60 and 70 in separate experiment columns as opposed to just 60 in one column).

Step 3: Enter dengue testing data (see Table 9 and section 1.3) stratified by children and adults.

	A	B	C	D	E	F	G
1							
2	1.3. Over-diagnosis analysis						
3							
4		Suggested range of values	Diagnostic test positive	Diagnostic test negative	**Total**	**PPV**	
5	Notified dengue (children)	0.5-0.95	1758	439	**2197**	**0.8**	
6	Notified dengue (adults)	0.4-0.9	1758	439	**2197**	**0.8**	
7							
8	* see Tables 7 and 8 for suggestions on what appropriate tests to include.						

If data disaggregated by children and adults are unavailable, enter all data in the "children" rows; the same values will be automatically assumed and calculated for adults.

Step 4: Enter over-diagnosis analysis data (see Table 10 and section 1.4) stratified by adults and children.

1.4. Under-diagnosis analysis

	Suggested range of values for Sensitivity	Experiment 1			Experiment 2			Experiment 3		
		True dengue cases in UFI sample	Dengue cases correctly diagnosed in dengue positive UFI sample	Sensitivity	True dengue cases in UFI sample	Dengue cases correctly diagnosed in dengue positive UFI sample	Sensitivity	True dengue cases in UFI sample	Dengue cases correctly diagnosed in dengue positive UFI sample	Sensitivity
Notified dengue (children)	0.5-0.95	1000	800	0.8	1000	700	0.7	1000	900	0.9
Notified dengue (adults)	0.4-0.9	1000	800	0.8	1000	700	0.7	1000	900	0.9

Enter data for multiple experiments in separate column groups.

Step 5: Enter fever cohort data (see tables 11–12, section 1.5) stratified by children and adults.

2.1. Febrile illness cohorts

Measure (within cohort)	Number (child cohort)	Number (adult cohort)	Suggested range of values
Person-years of observation (Number in cohort * years each were observed for)	800	0	
Confirmed apparent dengue infections	27	0	2-30 per 1000 cohort person years
Confirmed apparent dengue infections that sought treatment	22	0	0-15 per 1000 cohort person years
Incidence of notified dengue cases in the area of the cohort study per 100,000 residents	230	0	10-1000 per 100,000 residents
Apparent dengue cases per notified case	**14.67391304**	**14.67391304**	
Proportion of apparent cases that seek treatment	**0.814814815**	**0.814814815**	
Serological cohort study calculator			
Total dengue infections (primary and secondary, asymptomatic and symptomatic)	40	0	10-300 per 1000 cohort person years
Dengue infections per notified case	**21.73913043**	**21.73913043**	

If serological measures are part of the febrile cohort, also add seroconversion results.

Step 6: Enter seroprevalence and corresponding notified case data (see section 1.6).

2.2. Seroprevalence surveys

	Suggested range of values	Survey 1	Survey 2	Survey 3	Survey 4	Survey 5
Force of infection estimate (0-1)	0.05-0.25	0.141	0	0	0	0
Incidence of notified cases per 100,000 residents in area of the survey	100-10,000 per 100,000 residents	220	0.000	0.000	0.000	0.000
Predicted infection incidence per 100,000 residents	10-1000 per 100,000 residents	**6964.465**	-	-	-	-
Dengue infections per notified case	1.5-30 per notified case	**31.657**	-	-	-	-

Age group	National population
0-4	1439761
5-9	1483591
10-14	1525674
15-19	1646827
20-24	1591126
25-29	1340562
30-34	1290121
35-39	1258112
40-44	1170941
45-49	1030560
50-54	917139
55-59	671403
60-64	496177
65-69	404749
70-74	303479
75-79	359473
80-84	0
85-89	0
90-94	0
95-99	0
100+	0
Total	16929695

Also enter national population age distribution from census data in the lower table.

Step 7: Examine final burden estimates in the final sheet of the burden calculator (all automatically calculated).

	A	B	C	D	E
1					
2	FINAL ANNUAL BURDEN ESTIMATES				
3	Country-data estimated:				
4		Estimated burden			
5	Dengue disease severity	Low	Mean	High	
6	Death	95	95	95	
7	Severe dengue	3,556	3,556	3,556	
8	Non-severe dengue	47,035	56,026	68,032	
9	Self managed dengue	130,383	391,884	653,384	
10	Inapparent dengue	317,999	829,431	1,337,850	
11					
12	**Total dengue clinical burden**	**50,685**	**59,677**	**71,683**	
13	**Total dengue community burden**	**448,382**	**1,221,315**	**1,991,234**	
14					

Simple estimates of the upper and lower bounds of the final estimated burden are provided based on the upper and lower ranges of results of the different experiments or surveys.

If estimated values are used for any section, it is recommended that users conduct a series of experiments with different values for parameters for which they are uncertain and assess what affect these changes have on both the median burden estimate but also the upper and lower bounds of the prediction.

2.2 COMPARISON WITH GLOBAL DENGUE BURDEN MODELLING ESTIMATES

Model-based burden estimates use data from many different contexts in combination with epidemiological theory and risk factors for the disease to produce estimates of different types of dengue burden (**Table 14**). Each of these different modelling approaches has its own strengths and weaknesses, and each approach is more or less suited to estimating different levels of severity of dengue burden.

The burden estimates made using the burden calculator can be compared to each of these modelled burden estimates by selecting the relevant country in the brown "Select country" box.

Some of these burden estimation methods can also be used to provide improved parameter estimates for sections in the burden calculator for which there are no country-specific data (*26*).

Table 14. Summary of the different independent model-based burden global dengue burden methods

Institutional home:		Oxford	Global Burden of Disease	Brandeis
Main data sources		Serological cohort studies and environmental risk maps	Databases of all-cause mortality and dengue risk factors	Fever cohort studies and reported case numbers in selected countries
Further details (reference):		Bhatt et al., 2013 (*17*)	www.healthdata.org and Stanaway et al., 2016 (*27*)	Shepard et al., 2016 (*28*)
Estimates				
Year for which burden estimates were made		2012	2016	2013
Deaths		–	Yes (37,737)	Yes (13 586)
Apparent infections	Clinical	Yes (96 m)	Yes (101 m)	Yes (37.4 m)
	Non-clinical			Yes (21 m)
Deaths				Yes (13 586)
Inapparent infections		Yes (294 m)	–	–

FURTHER EXTENSION STUDIES

3.1 SPATIAL VARIATION IN BURDEN

Transmission of dengue is highly heterogeneous and national burden numbers are unlikely to reflect subnational variations. Generating more fine-scale predictions of dengue burden is important for developing targeted strategies that can improve the way limited surveillance and control efforts are deployed.

The required degree of spatial disaggregation in burden will depend on each country's dengue epidemiology and capacity to adopt different surveillance and control strategies in different areas. These are most frequently constrained to different levels of administrative divisions (Admin1/state/province, Admin2/municipality/county, Admin 3/neighbourhood).

Once the desired scale has been chosen, the first step should be to stratify each unit into a suspected risk category based on available epidemiological and entomological data. For example, if estimating burden at the state level, states may be categorized into high, medium or low suspected risk based on total notified case incidence estimates e.g. > 1000, 100–1000 or < 100 notified cases per 100 000 residents per year. The exact thresholds chosen should balance the need for an even number of units in each risk category with other epidemiological considerations, such as separating highly seasonal transmission units from those with year-round transmission.

The burden estimation approach detailed in this toolkit should then be repeated within each of these risk strata to give separate estimates of dengue burden in high, medium and low-risk states.

To obtain burden estimates at finer spatial scales (e.g. municipality or neighbourhood level), it is not practical to conduct the full programme of recommended burden estimation activities in every spatial unit. Instead, a regression approach is more suitable where administrative regions within the country are stratified into high, medium, or low transmission intensity categories based on historical dengue incidence and environmental or socioeconomic characteristics. The full range of burden estimation activities is then carried out in at least one of the high, medium and low administrative regions, with the results extrapolated to other regions in the same category. This method is explained in more detail in the WHO guidance on seroprevalence survey design (*22*). A number of potentially explanatory covariates that should also be collected from all units may explain variation in overall dengue incidence or in the way it is diagnosed and reported (Table 15). These covariates can typically be extracted from national census records or meteorological datasets.

Separate linear regression models should be created for each of the measured burden parameters measured in sections 1.2–4 and 2.1–2. Each of these models should be fitted with all combinations and permutations of ex-

planatory covariates to create a list of candidate models. The model with the lowest Akaike Information Criterion should be selected as the final model. This final model can then be used to make predictions in all units where a burden assessment did not take place.

For more detail on stratification, sample size estimation and the regression approach refer to the WHO guidance on seroprevalence survey design (*22*). More fine-scale mapping and burden estimation or assistance with this regression approach may require the assistance of a geospatial modeller.

Table 15. Examples of potentially explanatory covariates for subnational dengue burden estimation

Covariates for dengue transmission	Covariates for surveillance system quality
Number of notified cases	% of notified cases that are test confirmed
Rainfall	Measures of individual income
Temperature	Healthcare budget
Urbanization	Doctors per person

3.2 ECONOMIC BURDEN OF DENGUE

Estimation of dengue burden provides an opportunity to also consider the economic burden of the disease, which can be used to make the case for new investments in its surveillance and control.

The types of costs most relevant to the impact of dengue can be subdivided into direct and indirect costs. Direct costs include costs directly related to the treatment of an acute dengue illness episode and can include medical costs (e.g. medical care, diagnostic tests and medicines paid for by the public healthcare sector or the patient) and non-medical costs (e.g. cost of travel to seek treatment). Indirect costs represent losses of productivity (typically work wages) due to illness or premature death.

The direct and indirect cost of each dengue illness episode will vary depending on severity of illness. For the purpose of economic burden analysis, it may be more beneficial to re-categorize non-fatal dengue cases (non-severe dengue and severe dengue) by treatment setting (inpatient or outpatient) as it is a principle determinant of direct medical costs.

Methods for gathering cost data can be found by referring to previous studies (*29*). Once the cost per illness episode is derived, the total economic burden can be obtained by multiplying the cost per illness episode with the number of illness episodes at each level of severity (see **Table 16**). Further disaggregation or detail of economic burden may require collaboration with a health economist.

Table 16. Calculating the national economic burden of dengue

Type of case		Cost per illness episode	Number of illness episodes (from burden estimation)	Total cost
Short-term costs	Hospital (direct costs)			
	Hospital (indirect costs)			
	Outpatient (direct costs)			
	Outpatient (indirect cost)			
	Outside medical sector (indirect)			
Long-term costs	Fatal child (indirect)			
	Fatal adult (indirect)			
Total				

3.3 COMBINED ARBOVIRAL BURDEN

Estimating the burden of dengue presents an opportunity to also estimate the burden of other arboviral infections, such as Zika virus disease, chikungunya and yellow fever. Indeed, co-estimating the burden of Zika virus disease and chikungunya at the same time as that for dengue may improve dengue burden estimates due to the additional insight into misdiagnosis among arboviral diseases. This will be most relevant for countries that have experienced large-scale outbreaks of these diseases and have continued circulation of more than one arbovirus.

There is a growing recognition that clinical guidelines for diagnosis and management of arboviral infection need to change when dengue, chikungunya and Zika virus disease co-circulate due to the high potential of misdiagnosis and hence improper management. The Pan American Health Organization has published guidelines for differentiating dengue, chikungunya and Zika using clinical diagnosis (section 5) and laboratory testing (section 7) (*30*).

We recommend using these clinical and laboratory testing algorithms when performing the over and under diagnosis analyses (1.3 and 1.4) and febrile cohort (1.5) studies in areas of high prevalence of dengue, Zika and chikungunya.

If Zika virus disease is co-circulating, the inclusion criteria for arbovirus testing in the UFI study (under-diagnosis analysis, 1.4) and febrile cohort (1.5) should also include rash, even if the participants still attend school or work. Self-reporting of rash to study organizers should be encouraged in all cohort participants.

The remaining stages of the burden toolkit can be completed as normal, but with separate entries for each arbovirus.

3.4 REDUCING UNCERTAINTY IN BURDEN ESTIMATES

The value of burden estimation is not just to generate a single estimate of a country's burden but rather to calculate the uncertainty around this figure, identify the source of this uncertainty and target future data collection activities to improve the accuracy of future burden estimates.

Uncertainty in burden estimates from the burden calculator can come from two sources:

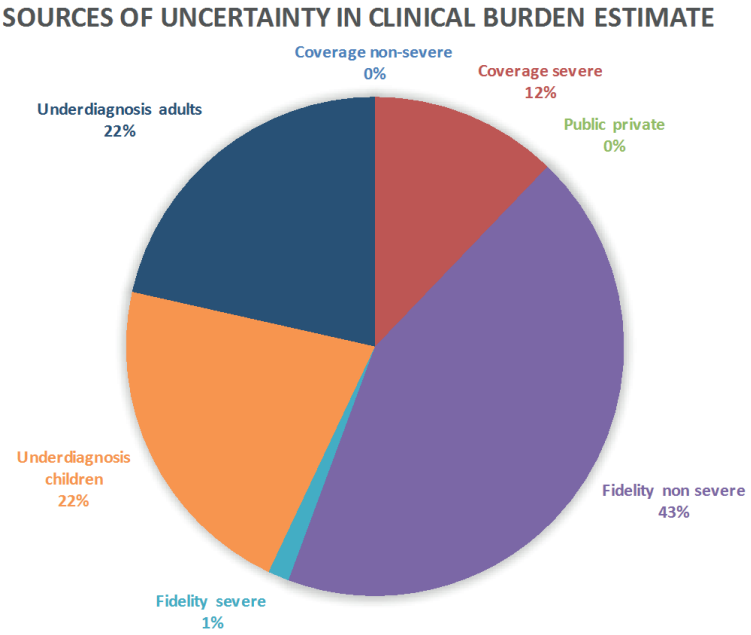
1. Parameter uncertainty, where two data sources of the same type suggest different values

e.g. two different reporting fidelity assessments suggest two different values for reporting fidelity

2. Method uncertainty, where two different methods for calculating the overall burden of a particular level of dengue severity give different answers

e.g. calculating apparent dengue incidence using (i) apparent cases per notified case times total notified cases or (ii) apparent cases per cases that sought treatment times total estimated clinical burden and the two methods give different answers

In the burden calculator tool, the clinical burden of dengue uses only one method, so is only affected by parameter uncertainty. By default, a pie chart of sources of parameter uncertainty is produced on the FINAL BURDEN ESTIMATION tab:



This pie chart can be used to prioritize future data collection efforts to reduce uncertainty in the clinical burden of dengue. In this example from Sri Lanka, the greatest reduction in uncertainty would be gained by conducting further fidelity assessments, particularly into the notification fidelity of non-severe dengue cases. This may include stratifying such assessments across urban versus rural healthcare provision environments to provide more locally-specific measures of notification fidelity.

Reducing estimates of the community burden of dengue is also important as it accounts for a larger proportion of total burden than clinical burden and is often measured using more limited data. As well as the parameter uncertainty in each of the measurements extracted from febrile cohorts and seroprevalence surveys, there is additional method uncertainty around how different data types are combined.

Estimates of the number of self-managed apparent dengue cases can be obtained by directly measuring the incidence of self-managed dengue in the febrile cohort, or by subtracting the estimated clinical incidence calculated using the burden calculator from the incidence of all apparent infection in the cohort. The former method has the advantage of direct measurement of self-managed dengue, but may not be generalizable to the whole country and treatment seeking behaviour may be very different in the area of the febrile cohort than in other parts of the country. The latter method is more generalizable, but assumes that all gaps in routine passive surveillance have been correctly enumerated in the burden calculator.

If these two methods produce conflicting results, the difference may be explained by varying treatment-seeking rates. Demographic and Health Surveys (DHS) routinely collect information on treatment seeking for fever and these should be examined to test the hypothesis of different treatment rates in the area of the febrile cohort. If this shows no difference, countries should attempt to conduct completeness analyses, over-diagnosis analyses and under-diagnoses analyses in the same catchment area as the febrile cohort to see if routine surveillance is more or less complete in that area.

The total number of dengue infections (which is also used to estimate the number of asymptomatic infections) can also either be estimated by serological febrile cohorts or seroprevalence surveys. The strengths and weaknesses of each approach are outlined in Table 13; however, both add value to burden estimation. If serological cohort studies were performed in years with abnormally high or low dengue incidence (as determined by comparison with routine surveillance data), this may explain why observed incidence is higher or lower than estimated by seroprevalence surveys that estimate long-term average incidence. The limitations of calculating force of infection from seroprevalence surveys should also be considered, and seroprevalence surveys that suggest implausibly high or low infection rates relative to notified cases should be examined.

3.5 EVALUATING CHANGE IN BURDEN OVER TIME

One of the ultimate aims of wide-scale burden estimation is to quantify the impact of interventions on disease morbidity and mortality at the national and international scales. Measuring such effects is often complicated by parallel improvements in disease surveillance that lead to more cases being reported. Measuring changes in surveillance systems is, therefore, important in assessing burden change over time (Table 17). Such changes can include, but are not limited to, changes in dengue testing procedures and methods, physician training for arbovirus diagnosis and greater provision of government subsidized healthcare. Measuring these changes will require re-applying the surveys detailed in this dengue burden

estimation toolkit (1.1–4. and 2.1–2) at periodic time intervals. Depending on the surveillance system, however, some of these surveys may be required to be completed more or less frequently to achieve reliable dengue burden estimates.

The most common increases in disease surveillance efforts are to (i) increase the completeness of routine surveillance systems (1.2) and (ii) increase the availability of dengue testing to support clinical diagnosis, which decreases over-diagnosis (1.3). If surveillance system completeness is already high and a high proportion of notified cases are already tested using appropriate dengue-specific diagnostic tests, updating the completeness assessment and over-diagnosis analysis are probably unnecessary; however, updating will be required if the national case definition for dengue changes.

The over-diagnosis analysis may also require reassessment at semi-frequent intervals, particularly if there are big increases in treatment or point-of-care diagnostic capacities that allow greater detection of early-stage clinically non-specific dengue cases. Furthermore, the over-diagnosis analysis should be re-performed if other major causes of febrile illness increase, e.g. Zika virus disease.

In the absence of any major nationwide intervention programmes or invasion of new dengue serotypes, the burden of DENV infection in the community is unlikely to change significantly. As a result, febrile illness cohorts and seroprevalence survey measures can be updated at less frequent intervals in many settings. Treatment-seeking behaviours (which are measured as part of the febrile illness cohorts) may change at more frequent intervals, particularly if major new sources of care become available, such as new private hospitals, or longer-term socioeconomic changes occur that increase utilization of healthcare services.

Follow-up seroprevalence surveys are likely to require a different experimental design and different data analysis techniques to inform changes in burden over time. Younger children must be enrolled in follow up seroprevalence surveys as observing changes in the past 5 years, for example, requires enrolling children aged below 5 years. Data must also be analysed using modelling methods that incorporate time-varying force of infection estimates.

Table 17. Frequency of burden estimation activity repeat measures to monitor changing dengue burden over time

Activity	Effort	Frequency	Considerations
1.1 Data assembly	Low	Annually	Changes in case definition will require repeating all of sections 1.2–4
1.2 Completeness assessment	Low	Annually	Unless already high
1.3 Over-diagnosis analysis	Low	Annually	Unless a high proportion of notified cases are already test-confirmed
1.4 Under-diagnosis analysis	Medium	Semi-frequently (~2 years)	May be required more frequently if new causes of febrile illness emerge, e.g. Zika virus disease
1.5 Febrile illness cohorts	High	Infrequently (~5 years)	Surveys of treatment-seeking behaviour may require more frequent updates
1.6 Seroprevalence surveys	High	Infrequently (~5 years)	Survey protocol and analysis requires modification to detect changes

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Dengue is a viral disease vectored by *Aedes* mosquitoes that has spread throughout the tropical world since the mid twentieth century. Existing reactive control efforts have failed to stop the expansion of dengue virus transmission and many areas now have endemic circulation of all four dengue virus (DENV) serotypes. New strategies are needed to reverse this trend; and to be effective, they must be based on accurate quantitative information about the burden of dengue.

The aim of this toolkit is to estimate the national annual burden of dengue when applied in a given country or subnational area. This is achieved through a series of six sub-objectives that: assemble existing data, amend gaps in surveillance completenes, and correct for both over and under diagnosis to estimate the true clinical burden of dengue. These clinical burden estimates should then be combined with new or existing community-based surveys to estimate the symptomatic and inapparent community-based burden of dengue.