

Malaria Elimination Toolkit

Entomological Surveillance Planning Tool (ESPT)

Malaria Elimination Initiative



University of California San Francisco

The Malaria Elimination Initiative is a project of the Global Health Group at UCSF Institute for Global Health Sciences.

shrinkingthemalariamap.org

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An operational decision support tool for national malaria programs to promote data-driven vector control.

Developed by the University of California, San Francisco, Global Health Group Malaria Elimination Initiative and the University of Notre Dame in collaboration with national malaria programs and an Entomological Surveillance Working Group with funding from the Bill & Melinda Gates Foundation.

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The Malaria Elimination Initiative (MEI) at the University of California San Francisco (UCSF) Global Health Group believes a malaria-free world is possible within a generation. As a forward-thinking partner to malaria-eliminating countries and regions, the MEI generates evidence, develops new tools and approaches, disseminates experiences, and builds consensus to shrink the malaria map. With support from the MEI's highly-skilled team, countries around the world are actively working to eliminate malaria – a goal that nearly 30 countries will achieve by 2020.

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About the Malaria Elimination Toolkit

The Malaria Elimination Toolkit is a set of proven tools and approaches to help malaria endemic countries accelerate progress toward malaria elimination. Developed by the Malaria Elimination Initiative (MEI) at the University of California, San Francisco (UCSF), the toolkit addresses the unique challenges faced by national malaria programs in heterogeneous transmission settings. These tools have been used successfully at the national and/or subnational levels, leading to important changes in malaria policy and practice.

The Malaria Elimination Toolkit focuses on three primary areas: situation assessment, tailored responses, and program management and sustainability – with the ultimate goal of building capacity and optimizing a country or district's ability

to advance toward elimination. To support situation assessment, the MEI has developed tools that help malaria programs understand the drivers of transmission in a target area and the readiness of the health system for elimination. MEI tools can further help a program decide what actions to take based on the situation assessment, how to tailor its response, and how to ensure efforts are well-managed and sufficiently funded.

The MEI recognizes that implementing any new tool or approach can be challenging. Technical assistance is available to support the tailoring and implementation of all MEI tools. Please visit our website <http://www.shrinkingthemalariamap.org> or contact us for more information: mei@ucsf.edu.



Situation assessment

What are the drivers of transmission?
What is the readiness of the health system for elimination and what are the gaps?



Tailored response

What actions should the program take based on identified and characterized gaps?



Program management & sustainability

How does the program effectively manage and fund malaria elimination?

The MEI Malaria Elimination Toolkit priority areas

Introduction

Entomological surveillance is essential for understanding vector species, specific population dynamics, and behavioral traits that affect disease transmission and intervention effectiveness over time. Entomological surveillance data should guide intervention selection, targeting and tailoring of interventions, and deployment in space and time, and can provide a framework to evaluate complementary strategies and tools. In areas of plateauing or increasing malaria transmission, entomological surveillance may help identify potential drivers of transmission. In low transmission settings, entomological surveillance is a critical part of foci investigation to inform foci response and eliminate pockets of remaining transmission. In communities working to prevent reestablishment of malaria transmission, entomological surveillance is useful for monitoring changes in receptivity that could enable reestablishment of transmission with imported parasites. Moreover, as heterogeneous transmission is present in most places, tailoring vector control interventions to address the local problem and targeting these interventions to the appropriate population group, in the relevant place, at the right time, is increasingly critical to successfully reduce malaria transmission.

Understanding why and where transmission is persisting, ensuring effective vector control, and monitoring trends are critical to accelerating progress toward malaria elimination. In this context, the role of entomological surveillance is more important than ever.

The World Health Organization (WHO) Global Technical Strategy 2016–2030 outlines five core vector control elements to accelerate progress:¹

1. Maximize the impact of vector control
2. Maintain adequate entomological surveillance and monitoring
3. Manage insecticide resistance and residual transmission
4. Strengthen capacity for evidence-driven vector control
5. Implement malaria vector control in the context of integrated vector management

1 WHO. Global technical strategy for malaria 2016–2030. World Health Organization. Geneva. 2016.

Entomological surveillance is central to all five elements. The WHO Global Vector Control Response further emphasizes the need for effective, locally adapted, and sustainable vector control based on increased capacity and enhanced entomological surveillance.² Further guidance on entomological surveillance is provided in the WHO Malaria Surveillance, Monitoring and Evaluation: a reference manual that includes entomological surveillance requirements at different levels of malaria transmission.³

This Entomological Surveillance Planning Tool (ESPT) aligns with and aims to distill WHO guidance into a decision-support tool for malaria programs to strengthen entomological surveillance and support cost-effective, locally tailored, evidence-based vector control. As such, the ESPT supports malaria programs to target and tailor vector control interventions. The ESPT also incorporates guidance from the President's Malaria Initiative (PMI) and other technical partners and resources. The updated Malaria Eradication Research Agenda (malERA) highlighted the need for minimal essential entomological data that is **collectable and actionable** for national malaria programs,⁴ and the ESPT responds to this call.

Given that the pathway toward elimination is a continuous process and not a set of independent stages,¹ the ESPT prioritizes entomological surveillance indicators and activities across transmission settings, geographic areas (sentinel sites versus transmission foci), and levels of program capacity. The ESPT considers how these indicators and activities influence national malaria program decisions about entomological surveillance planning and vector control response.

The ESPT was developed in direct response to national malaria program demand for more operational guidance in entomological surveillance. An Entomological Surveillance Working Group (ESWG) of experts from national malaria programs, regional elimination networks, WHO, PMI, academia, and implementing partners has contributed to the design

2 WHO. Global vector control response 2017–2030. World Health Organization. Geneva. 2017.

3 WHO. Malaria surveillance, monitoring & evaluation: a reference manual. World Health Organization. Geneva. 2018.

4 Rabinovich, RN, et al. (2017) malERA: An updated research agenda for malaria elimination and eradication. *PLoS Medicine*; 14(11): e1002456.

and development of the ESPT. The development of the ESPT was led by the University of California, San Francisco, Global Health Group's Malaria Elimination Initiative (MEI) and the University of Notre Dame with funding from the Bill & Melinda Gates Foundation.

What is the ESPT?

The ESPT is a decision-support tool for planning entomological surveillance activities, interpreting and integrating entomological data with epidemiological data, and guiding programmatic vector control strategies. The ESPT includes practical approaches and minimum essential indicators to help answer programmatic questions about local transmission drivers, gaps in protection with current vector control interventions (e.g., insecticide resistance, outdoor biting, etc.), and how to select supplemental vector control interventions to address gaps. In turn, this data, in combination with epidemiological, intervention, and other data, will help malaria programs target and tailor vector control solutions, reduce vector populations and human-vector contact, and drive down transmission. Critically, the ESPT also includes indicators and methods to improve understanding of **human behavior** as it relates to increased exposure to infectious mosquito bites and **high-risk populations (HRPs)** that may be contributing to transmission but not accessing malaria preventative and treatment services.

Who should use this tool?

This ESPT is for national malaria program managers, vector control officers, program entomologists, surveillance officers, and M&E officers to use in collaboration with their implementing, technical, and research partners. The ESPT is also for individuals involved in planning entomological surveillance activities and interpreting entomological surveillance data at provincial and district levels. Technical assistance is available to support the tailoring and implementation of all MEI tools. Please visit our website and contact the MEI for more information: <http://www.shrinkingthemalariamap.org/contactus>.

How is this tool used?

This ESPT can be used in the following ways, depending on the needs of the program:

1. **Annual entomological surveillance planning.**
2. **Training framework for entomological surveillance.**

3. **Field and laboratory data collection.** While not a data collection tool, the ESPT offers a step-by-step guide about the field and laboratory data required to answer priority questions. The ESPT also provides specific guidance on selecting field sampling methods, which is critical to answering priority questions appropriately and efficiently.
4. **Framework for integration and joint analysis of entomological and epidemiological data.** The ESPT can be used as a framework for integrating entomological data with epidemiological data and other data (e.g., intervention, rainfall, etc.) to more comprehensively inform vector control decision making. This framework can then guide development of databases or platforms for integrating and visualizing data.
5. **Programmatic transmission investigations.** The ESPT can be used by malaria programs to design outbreak, foci, or other investigations to understand why there is ongoing malaria transmission in specific areas.
6. **Intervention evaluations.** The ESPT can be used to evaluate existing vector control interventions within a program and make decisions about changing current strategies and/or introducing new or supplemental interventions.
7. **Technical and resource capacity gap analysis.** As the ESPT guides malaria programs through entomological surveillance planning, the ESPT highlights the capacity needed to collect data on specific indicators and, in doing so, can help malaria programs to prioritize capacity development targets and identify technical and resource gaps that could be addressed by partnering with implementation partners and/or research collaborators.

The MEI in collaboration with national malaria programs and partners piloted an early draft of the ESPT in four countries across Mesoamerica, southern Africa, and the Greater Mekong Subregion. That draft was also shared and used independently (i.e., without support from the MEI) by malaria programs in other countries for national entomological surveillance training and strategy development. This version of the ESPT is a result of the pilot evaluations, feedback from malaria programs, and guidance from an Entomological Surveillance Working Group mentioned above to improve the content, utility, and usability of the tool.

How do I navigate this tool?

Figure 1 describes how to navigate through the ESPT. First, the user should determine the question(s) to be answered through entomological surveillance, e.g., where is transmission occurring? Is indoor residual spraying (IRS) working effectively against local vectors? What are the minimum essential entomological surveillance activities to make vector control decisions? **Module 1** will guide the user to formulate their question(s). **Module 2** guides the user through determining the indicators necessary to answer the question(s). **Modules 3, 4 and 5** provide guidance on sampling methods, site selection, and sampling design. These decisions must be made in the context of available capacity and resources, including human and financial. **Module 6** offers an approach for managing entomological data.

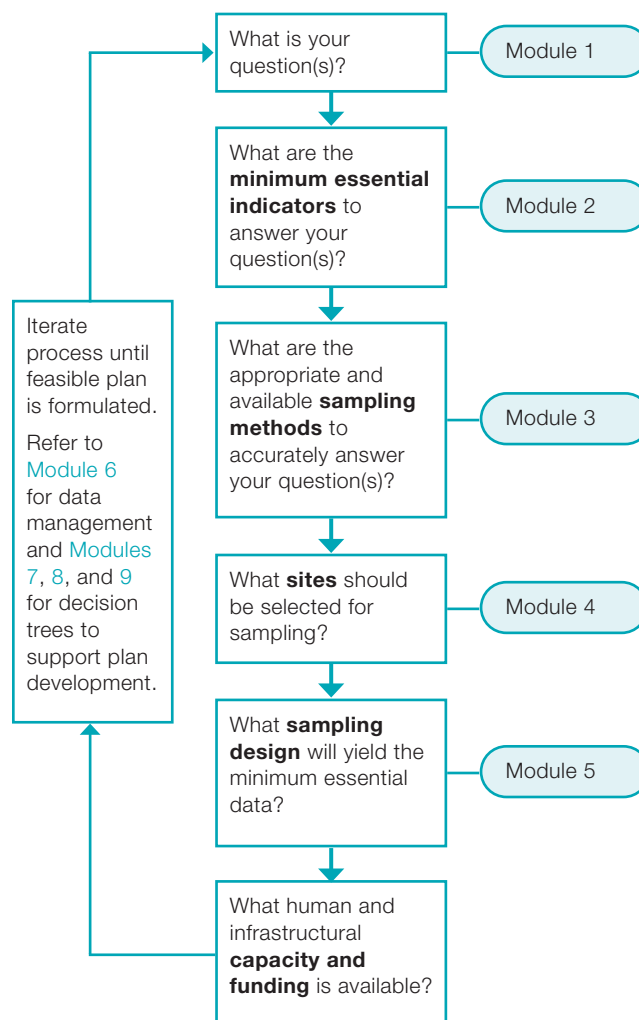
Modules 7, 8, and 9 include decision trees that build on previous modules to guide users to answer their priority question(s) and problem-solve by indicator and type of survey (see also **Module 4** for more on survey types):

- **Module 7** provides decision trees by indicator for baseline surveys that can also be used for spot surveys and for reference during routine surveys and foci investigation.
- **Module 8** includes decision trees by indicator for routine surveys to monitor trends over time, and identify and respond to changes, including in areas preventing reestablishment of transmission.
- **Module 9** offers decision trees for entomological surveillance during foci investigation in low transmission settings.

Six annexes support **Modules 1-9** and should be referenced accordingly:

- **Annex I:** Three examples of how the ESPT could be used to answer specific questions.
- **Annex II:** A specific decision tree for selecting LLINs based on insecticide resistance data.
- **Annex III:** Further description of entomological sampling methods and analytical techniques expanding on **Module 3**.
- **Annex IV:** An example of a data collection form for collecting data on human behavior.
- **Annex V:** A glossary of terms.
- **Annex VI:** A summary of supplementary vector control interventions and WHO recommendations.

Figure 1. Navigating the ESPT



Key ESPT Messages

1. To reduce malaria burden and achieve elimination, a **shift in mindset** is needed to identify gaps and drivers of transmission at the local level, and target and tailor solutions accordingly. The ESPT supports this **targeting and tailoring**.
2. The ESPT aims to support **program ownership** for entomological surveillance activities and vector control decision-making.
3. Entomological surveillance should be seen as a **core programmatic activity** by ministries of health and research, and ethics committees.
4. Entomological surveillance can be resource intensive, including labor, technical expertise, and advanced analytical equipment so **collaboration with both research and implementation partners** is key.
5. **Human behavior** is a central component of the ESPT, emphasizing that vector control should be targeted to the human-vector contact point (where transmission occurs).
6. The ESPT identifies opportunities to **integrate epidemiological data** with entomological data to guide action.
7. The ESPT helps to identify **gaps in protection**, or limitations with current prevention measures; for example, outdoor biting where no outdoor protection exists or insecticide resistance that limits the effectiveness of an insecticide-based intervention.
8. The ESPT highlights that entomological surveillance should be **iterative and adaptive** since malaria transmission is dynamic; constant adjustments should be made to improve sampling methods, design or analysis. This will ensure program questions are being answered appropriately and evidence-based decisions can be made and monitored.

Module 1. Identify Your Questions

For entomological data to be useful for malaria program decision-making, data should be collected with a specific programmatically relevant question(s) in mind, such as, what is driving an increase in transmission in a specific area? Or, are local vectors still susceptible to insecticide(s) currently used for indoor residual spraying (IRS) in a specific area? Some questions should be answered with data collected over time using baseline or routine surveys (see [Module 4](#)), while other questions can be answered with time-bound, spot surveys that target a specific area(s) with a particular question in mind. Some questions may be specific to foci of transmission while others may be best addressed by data collected across a representative set of sentinel sites (see [Module 4](#)).

Epidemiological data should also help trigger questions. For example, if a review of epidemiological data reveals that most malaria cases are men between the ages of 15 and 50 years, then it is possible malaria risk is associated with occupation

(that can be validated using methods outlined in [Box 3](#) in [Module 5](#)), which should drive the question: where are men between the ages of 15 and 50 years being exposed to possibly infective mosquito bites? This may trigger the need for entomological investigations in forest work sites, for example.

Another approach would be to start with a particular decision that your malaria program needs to make. For example, if a long-lasting insecticide-treated net (LLIN) procurement is approaching, the question of whether to procure a pyrethroid-only net versus a pyrethroid + PBO net or dual active ingredient (dual AI) net may arise. In this case, there may be specific entomological investigations to conduct to inform the procurement decision.

Below are examples of questions that emerged from pilot evaluations of this ESPT, as well as other frequently asked questions from national malaria programs.

Theme of question	Example question*
Performance of current vector control interventions (e.g., LLINs, IRS)	<p>*The questions could be applied nationally or sub-nationally (i.e., to a district or subset of districts) and in sentinel sites and/or transmission foci and/or other targeted areas.</p> <ul style="list-style-type: none"> • If no data existed previously, what is the baseline vector composition, distribution, and bionomics for further monitoring where interventions are currently deployed? • How do current interventions affect vector populations and malaria epidemiology over time? I.e., do current interventions result in a change in vector behavior and/or a reduction in vector populations, human-vector contact, and malaria incidence? • Are local vectors susceptible to current insecticide-based interventions? • How long is the quality and efficacy of current interventions sustained over time?
Selection and evaluation of supplemental interventions	<ul style="list-style-type: none"> • What is the baseline vector composition, distribution, and bionomics prior to introduction of an intervention? • What are the gaps in protection (e.g., outdoor vector biting) and what are the available interventions that can address those gaps? • Where and when should a supplemental intervention (e.g., larval source management (LS)) be deployed? • How do vector populations (e.g., behavior and species composition) change following introduction of a supplemental intervention?

Drivers of transmission in an area experiencing an increase or plateau of malaria cases	<ul style="list-style-type: none"> • What are potential entomological drivers of transmission? I.e., is local vector behavior and/or species composition and/or susceptibility to insecticides associated with an increase or plateau in malaria transmission? • How does human behavior affect intervention acceptance and usage and exposure to vector biting that could be driving transmission? • What is the association between vector populations, rainfall, and malaria incidence, and how can that association inform timing and targeting of interventions?
Changes in receptivity in an area trying to prevent reestablishment of transmission	<ul style="list-style-type: none"> • How do vector populations change over time in areas trying to prevent reestablishment? • How do these changes increase potential for transmission if imported parasites were introduced? • What action should be taken to address critical changes in receptivity?
Reduction or plateau in funding availability and/or capacity	<ul style="list-style-type: none"> • What are the priority entomological surveillance activities given a flatlining (or reduction) of funding and available capacity? I.e., what are the minimum essential indicators that should be collected to sufficiently inform vector control strategy? • What entomological surveillance activities can be conducted with current program capacity? What additional activities could be conducted with support from research or implementation partners?
Practical questions for enhancing and tailoring entomological surveillance activities	<ul style="list-style-type: none"> • Can CDC light traps serve as a valid proxy for human landing catches (HLCs) in a specific area? • Can <i>Anopheles</i> Species X be successfully reared in an insectary setting for insecticide resistance testing? • What method is most effective for collecting indoor resting mosquitoes in a specific area: pyrethrum spray catches (PSCs) or indoor aspirations?

The question or questions should then guide planning. To this end, the modules below provide operational guidance to malaria programs and their partners on planning entomological surveillance activities based on the program's question(s) and interpreting and integrating entomological data for decision making.

Module 2. Select Minimum Essential Indicators

Malaria programs could collect a lot of data if resources were available, but what is the minimum essential data required to make a program decision given finite resources? Below, [Table 1](#) is a list of minimum essential entomological indicators adapted from the WHO Malaria Surveillance, Monitoring and Evaluation manual. Included is a justification for each indicator and how each indicator informs decision-making. [Table 2](#) describes supplemental entomological indicators for programs to consider based on their relevance to decision-making and the available capacity and resources. [Table 3](#) provides additional indicators related to intervention effectiveness, and [Table 4](#) includes indicators relevant to human behavior and exposure risk.

Indicators require:

- Correct identification of species collected
- Correct documentation of collection site (including GPS coordinates if available) and date of collection
- Well defined and standardized denominators (e.g., number of collection nights and number of collectors or sampling devices per site)
- Standardized data collection across sites

Table 1. Minimum essential entomological indicators (by vector species, site, and date of collection)

Indicator	Outcome(s)	Significance
Adult vector composition and distribution		
Occurrence	Adult female vectors present or absent	This is important to 1) know whether your site is receptive to malaria transmission and 2) detect invasive species. This indicator can also be used to 3) determine vector species composition and seasonality and 4) monitor the impact of vector control interventions on specific vector species.
Density	Number of adult female vectors collected, usually per sampling method and unit time	This is important to 1) monitor the impact of vector control interventions on vector populations, 2) determine relative vector species composition, and 3) describe seasonality of vector populations.
Seasonality	Changes in vector species occurrence and density by season	This is important to inform appropriate timing of vector control interventions in combination with epidemiological and rainfall data.
Adult vector behavior		
Human biting rate (HBR)	Number of adult female vectors that attempt to feed per person per unit time	This is important to monitor both the potential for and impact of vector control interventions on human-vector contact and transmission. See how to calculate Adjusted Human Biting Rate in Table 4 to combine vector biting behavior and human behavior.
Biting time	Number of adult female vectors that attempt to feed per person per unit time	This is important to 1) identify gaps in protection combined with human behavior data and 2) target vector control interventions.

Biting location	Proportion of attempted bites or successful blood-feeds by adult female vectors indoors and outdoors per unit time	<p>This is important to 1) identify gaps in protection combined with human behavior data and 2) target vector control interventions.</p> <p>Simultaneous use of the same sampling method(s) indoors and outdoors is important for an indication of endophagy and exophagy.</p>
Indoor resting density	Proportion of adult female vectors collected resting indoors in structures sampled, usually per hour	<p>This is important to target and monitor vector control interventions. This indicator is especially relevant to evaluate 1) whether IRS might be effective and 2) how IRS is performing.</p>
Adult vector insecticide resistance		
Resistance frequency	Proportion of adult female vectors alive after exposure to insecticide	<p>This is important to monitor effectiveness of insecticide-based vector control interventions.</p> <p>It is important that the same vectors that rest and/or bite indoors are analyzed for resistance because those are the vectors targeted by LLINs and IRS.</p> <p>This includes using a discriminating concentration and time (i.e., diagnostic time) of insecticide in a standard bioassay.</p>
Resistance status	Classification of adult female vector populations as confirmed resistant, possibly resistant, or susceptible.	<p>This is important to inform decisions on vector control interventions and insecticides.</p> <p>Using a discriminating concentration of insecticide in a standard bioassay. <90% = confirmed resistance; 90–97% = possible resistance; ≥98% = susceptibility</p>
Immature vectors		
Larval habitat availability	Number of aquatic habitat present and absent, by area, habitat type, and season	<p>This is important to inform planning for larval surveys and LSM interventions.</p>
Larval habitat occupancy	Larvae and pupae present and absent by area, habitat type, and season	<p>This is important to 1) provide information on habitat preference, larval presence, and seasonality to inform LSM targeting and timing and 2) monitor receptivity in combination with adult vector occurrence and rainfall data.</p>
Transmission potential		
Receptivity	Classification of areas according to transmission risk	<p>This is important to measure and monitor potential for transmission in combination with parasite importation risk (i.e., vulnerability).</p> <p>Receptivity is a function of the presence of competent <i>Anopheles</i> vectors, a suitable climate, and a susceptible human population. Definitions and indicators are under review by the WHO.</p> <p>For purposes of this document, receptivity indicators include adult vector occurrence and larval habitat occupancy.</p>

Table 2. Supplemental entomological indicators (by vector species, site, and date of collection) based on relevance to the question and available capacity and resources

Indicator	Outcome(s)	Comment
Human blood index (HBI)	Proportion of blood-fed adult female vectors that feed on humans	This is useful to 1) determine anthropophagy and zoophagy of vectors and 2) target vector control interventions. Requires advanced laboratory capacity for blood meal analysis (i.e., ELISA).
Host preference	Proportion of adult female vectors collected feeding on humans or animals, of total vectors collected through human <i>and</i> animal baited sampling methods	This is useful to 1) determine anthropophagy and zoophagy of vectors and 2) target vector control interventions. Requires both human and animal-baited sampling techniques but no advanced laboratory capacity for blood meal analysis.
Larval density	Number of immature vectors collected per dip, per person, per unit time, by individual habitat	This is useful to 1) inform LSM targeting and 2) as a process indicator for monitoring LSM interventions. This is supplemental (not essential) because LSM decisions should be based on larval habitat occupancy. Usually reported by state category: early instar – stage I–II, late instar – stage III–IV, pupae.
Resistance intensity	Classification of adult female vector populations as having high, moderate, or low resistance	This is useful to 1) determine the level of insecticide resistance and 2) inform decisions on insecticide-based vector control interventions. Requires sufficient mosquito numbers for testing. Based on exposure to 5 x and 10 x intensity concentrations of an insecticide in a standard bioassay.
Resistance mechanism (synergist bioassay)	Difference between the proportion of dead or incapacitated adult vectors after exposure to insecticide + synergist and those exposed to insecticide alone	This is useful for an initial characterization of metabolic resistance. This indicator is especially relevant to inform procurement decisions about PBO LLINs.
Resistance mechanism(s) (molecular or biochemical tests)	Mechanism detected or not detected in adult female vectors	This is useful to 1) further characterize metabolic resistance and 2) monitor vector control interventions, including PBO LLINs. Requires advanced laboratory capacity.
Proxies for transmission		
Sporozoite rate (SR)	Proportion of adult female vectors with sporozoites in their salivary glands	This is useful to 1) identify <i>Anopheles</i> species capable of transmitting <i>plasmodium</i> and 2) estimate the proportion of <i>Anopheles</i> vectors present that are considered infectious. This indicator is difficult to measure and too resource intensive in low transmission settings and thus not recommended in these settings.

Entomological inoculation rate (EIR)	Number of infectious bites by adult female vectors per person per unit time, usually per year	<p>This is useful to 1) estimate level of transmission and 2) evaluate the impact of interventions.</p> <p>This indicator may be difficult to measure and resource intensive in low transmission settings and thus not recommended in these settings.</p> <p>It is not accurate to measure EIR during rainy season and extrapolate to a yearly EIR due to seasonal differences in mosquito densities and sporozoite rate.</p>
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Table 3. Indicators for monitoring performance of vector control interventions

Indicator	Outcome(s)	Comment
ITN/LLIN durability	<p>Survivorship (i.e., attrition)=total LLINs present in household at time of survey of total LLINs at distribution, over time</p> <p>Fabric integrity=proportionate holes index (pHI) per net based on number and size of holes</p> <p>Bio-efficacy=proportion of susceptible mosquitoes alive 24 hours post exposure by species</p>	This is important to 1) monitor effectiveness of nets and 2) identify gaps in protection if nets lose physical integrity and chemical efficacy.
ITN/LLIN access	<p>Proportion of people with access to an ITN/LLIN in their household OR</p> <p>Proportion of households with at least one ITN/LLIN for every two people</p>	This is important to 1) monitor access to ITNs/LLINs and 2) indicate whether there are gaps in protection as a result of lack of access to ITNs/LLINs.
ITN/LLIN usage	Proportion of people who slept under an ITN/LLIN the previous night	This is important to identify gaps in protection comparing use or non-use of ITNs/LLINs (human behavior) and vector behavior indoors.
ITN/LLIN use:access ratio	The proportion of the population using ITNs/LLINs, among those who have access to ITNs/LLINs within their household (divide use by access)	This ratio provides an estimate of the proportion of the population using nets, among those who have access to one within their household. This indicator clarifies whether a gap in net use is related to behavior or to lack of access to nets.
IRS residual efficacy	Proportion of susceptible vectors knocked down within 30 minutes while exposed to a sprayed wall or proportion of susceptible vectors dead within 24 hours (or 7 days for neonicotinoids) after being exposed to a sprayed wall (measured over the expected period of insecticide efficacy) by species and wall type	This is important to 1) monitor effectiveness of IRS and 2) identify gaps in protection if IRS efficacy does not extend through the malaria season(s), requiring an additional spray round or shift of the IRS campaign.
LSM effectiveness	Change in adult (species-specific) vector density after implementation	<p>This is important to monitor effectiveness of LSM intervention(s).</p> <p>Note the indicator is a change in adult vector density, not larval density, because adult densities is a better indicator of the impact of LSM on vector populations.</p>

Intervention coverage	Proportion of unit (e.g., person, house, larval habitat) with an intervention of total units	<p>This is important to monitor delivery of vector control interventions, and should be standardized across sites/country.</p> <p>This indicator is especially relevant in foci investigations to inform intervention top-ups/mop-ups.</p>
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Table 4. Indicators for measuring human behavior and associated risk factors

Indicator	Outcome(s)	Comment
<p>Sleeping or awake time by location</p> <p>(See Annex IV for sample data collection forms with data inputs)</p>	Proportion of individuals asleep vs. awake, indoors vs. outdoors by hour during biting times	<p>This is useful for analyzing vector behavior with human behavior and for determining where and when humans are potentially being exposed to mosquito bites.</p> <p>This indicator can be applied across time and relevant geographical areas to track population movement (e.g., sleeping in villages vs. sleeping at farms).</p> <p>Ideally measurements are made during the same periods and locations as vector biting measurements.</p> <p>It is important to note LLIN use every hour and/or whether walls were recently sprayed so that human and vector behavior can be analyzed with LLIN use/non-use and/or IRS status. This will help to identify gaps in protection. (See Example 2 in Module 7.)</p>
Adjusted human biting rate	Human biting rate x proportion of humans observed inside vs. outside, awake vs. asleep with or without a LLIN	<p>This is useful to analyze human behavior together with vector behavior and use of vector control interventions, described further in Example 2, Module 7. For example, proportion of vector bites occurring indoors for an unprotected individual vs. proportion of vector bites occurring outside for an unprotected individual.</p> <p>This indicator provides an idea of exposure risk and is especially useful when characterizing residual transmission in a programmatic context.</p>
<p>Malaria risk factors</p> <p>(See Box 3 in Module 3 for information on the High Risk Population Toolkit)</p>	Risk factors identified	<p>This is useful to inform targeting of vector surveillance and control, among other malaria services.</p> <p>Risk factors can include occupational exposures and other behaviors outside of households (e.g., forest-going, farming, cooking, etc.). Mobility of individuals and/or population groups may greatly vary (daily, weekly, seasonally), which in turn may affect the impact and effectiveness of vector control interventions, and consequently malaria risk. See Box 3.</p>
Parasite importation risk (i.e., vulnerability)	Frequency of influx of infected individuals or groups	This is useful to estimate transmission potential in combination with receptivity.

Important datasets for integrated analyses.

Analyzing entomological data alone will rarely tell the whole story or show the whole picture. The same can be said for epidemiological data. Instead, entomological and epidemiological data should be analyzed together to identify relationships and trends and to inform intervention selection and targeting.

Below is a list of key datasets for integration into analysis, visualization, and decision-making:

Malaria incidence by week or month by unit (health facility and/or district and/or village) corresponding as close as possible to the site(s) for entomological surveillance.

Malaria cases by case classification (as available), including indigenous and imported

- Mean and/or total rainfall by week by site
- Changes in receptivity and/or importation risk (i.e., vulnerability), including new construction sites, population movement for harvesting season, etc.
- Availability of malaria diagnosis and treatment, including antimalarial stockouts.

These factors can all be considered possible “drivers” of transmission, along with entomological drivers (e.g., outdoor biting) and should be included in any analysis and interpretation of entomological data and indicators. The decision trees included in this ESPT provide examples of how this can be done.

Understanding how vector control interventions function. To select the appropriate indicators to answer program questions, it is important to understand how vector control interventions exploit vector biology. Although not an exhaustive list, [Table 5](#) describes the vector behavioral traits that are targeted by various vector control interventions and [Figure 2](#) illustrates at what point these interventions function in the vector life cycle.

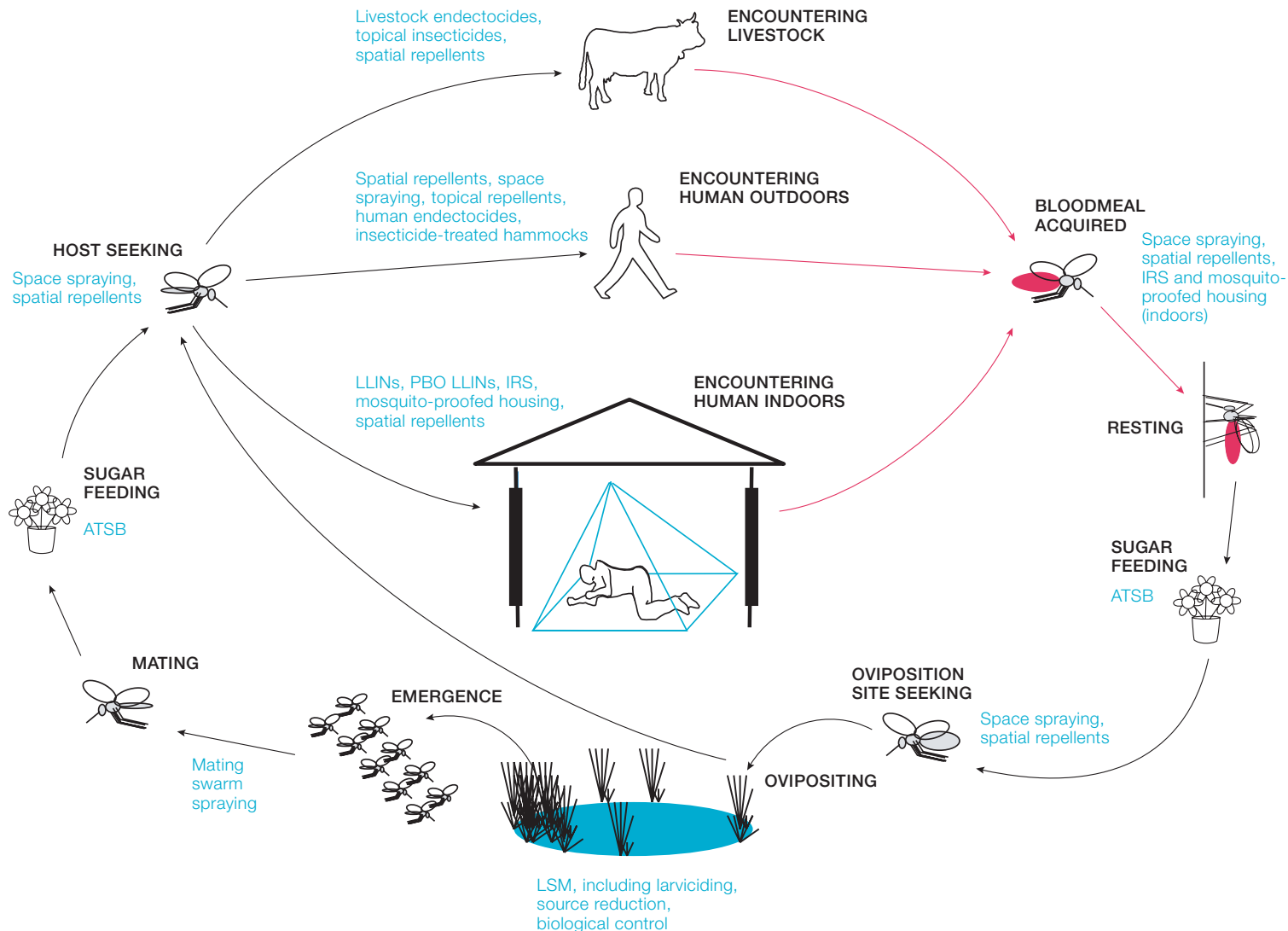
Table 5. Vector behavior targeted by select interventions

Intervention	Vector behavior targeted by intervention
LLINs	Insecticide susceptible indoor, late night human biting vectors (when people use LLINs)
PBO LLINs	Oxidase-based metabolic resistant indoor, late night human-biting vectors (when people use PBO LLINs)
Insecticide treated hammocks	Insecticide susceptible human-biting vectors (when people use hammocks)
IRS	Insecticide susceptible indoor resting vectors
LSM	Productive habitats for immature vectors
Insecticide-treated house materials/modifications	Insecticide susceptible, indoor (or structure) entering vectors
Non-insecticide housing materials/modifications	Indoor (or structure) entering vectors
Space spraying (outdoor)	Insecticide susceptible, host and sugar-seeking, and outdoor resting vectors
Spatial repellents	Insecticide susceptible, host and sugar-seeking, and resting vectors
Topical repellents (applied to humans)	Insecticide susceptible, anthropophagic vectors
Attractive targeted sugar baits (ATSBs)	Sugar-seeking vectors
Human endectocides	Anthropophagic vectors
Livestock endectocides	Zoophagic vectors

[Figure 2](#) describes the *Anopheles* mosquito life cycle and the specific points where interventions intervene to kill or repel mosquitoes, taking advantage of specific vector behaviors as outlined in [Table 5](#) above.⁵

5 Graphic adapted from a graphic in Kiware SS, Chitnis N, Tatarsky A, et al. Attacking the mosquito on multiple fronts: insights from the Vector Control Optimization Model (VCOM) for malaria elimination. *PLoS ONE*. 2017;12(12).

Figure 2. Understanding how vector control interventions target different stages of the *Anopheles* mosquito life cycle*



Tables 6 and 7 on the next pages note the minimum essential indicators needed to determine whether a *new* intervention should be introduced (Table 6) and those needed to determine whether an *existing* intervention is working effectively (Table 7). As explored further in the ESPT, note that some sampling methods may be able to capture data for multiple indicators simultaneously (e.g., human landing catches (HLC)).

*Kiware SS, Chitnis N, Tatarsky A, Wu S, Castellanos HMS, et al. (2017) Attacking the mosquito on multiple fronts: Insights from the Vector Control Optimization Model (VCOM) for malaria elimination. PLOS ONE 12(12): e0187680. <https://doi.org/10.1371/journal.pone.0187680>

Table 6. Minimum indicators to determine whether an intervention may be effective at a given site in a programmatic setting

	LLINs	PBO LLINs	Insecticide treated hammocks	IRS	LSM	Insecticide-treated housing materials/modifications	Non-insecticide housing materials/modifications	Space spraying	Spatial repellents	Topical repellents	ATSBs	Human endectocides	Live-stock endectocides
Minimum essential indicator (by species by site)													
Vector occurrence													
Vector density													
Seasonality (adult vectors)													
Larval habitat availability													
Larval habitat occupancy													
Human biting rate													
Biting time													
Biting location													
Indoor resting density													
Resistance frequency											Depends on active ingredient		
Resistance status											Depends on active ingredient		
Supplemental indicator (by species by site where relevant)													
Human blood index													
Host preference													
Resistance mechanism (synergist bioassay) (PBO)													
Human sleeping time													
Human sleeping location													



Yes



Depends



Resistance to larvicides (e.g., temephos)

*

Table 7. Minimum indicators to evaluate whether an intervention (already in place) is effective in a programmatic setting

	LLINs	PBO LLINs	Insecticide treated hammocks	IRS	LSM	Insecticide-treated housing materials	Non-insecticide housing materials	Space spraying	SRs	Topical repellents	ATSBs	Human endectocides	Livestock endectocides
Minimum essential entomological indicator (by species by site).^a													
Malaria cases will be the primary indicator for observing the impact of interventions, with consideration of other direct and indirect factors													
Vector occurrence													
Vector density													
Larval habitat density					b								
Larval habitat occupancy													
Human biting rate													
Biting time									d				
Biting location													
Indoor resting density													
Resistance frequency					c						Depends on active ingredient		
Resistance status					c						Depends on active ingredient		
Supplemental indicator (by species by site as relevant)													
Larval density													
Human blood index													
Host preference													
Resistance mechanism (synergist bioassay)													
ITN/LLIN durability													
ITN/LLIN usage													
IRS residual efficacy													
LSM effectiveness													
Intervention coverage													

	Yes
	Depends

a. Minimum essential indicators may vary depending on the question and study design. Use this table as a helpful guide to selecting indicators which are most essential to answer your question. b. Environmental management only. c. Larvicide resistance (e.g., temephos). d. Product dependent.

Module 3. Select Sampling Methods and Analytical Techniques

Key Messages

1. Every sampling method comes with a bias. Understanding the bias is critical to using the method appropriately.
2. Many sampling methods require local evaluation when being used for the first time to test sensitivity and specificity of the method.
3. Select sampling methods based on the question you are trying to answer.
4. When using multiple methods, consider the interaction between methods.
5. Well-designed sampling can capture data for multiple indicators and/or to answer multiple questions using the same methods.
6. Consistent quality control of entomological sampling is an essential component of entomological fieldwork to ensure reliability and robustness of entomological data collected.

Entomological sampling methods take advantage of specific mosquito behaviors, and each method has its own biases, advantages, and disadvantages. Selecting the appropriate sampling method and its placement (location and time) is critical to collecting relevant and accurate data. For example, a human-baited trap (e.g., CDC light trap hung near a human) placed inside houses may function very well with only indoor biting (endophagic) and anthropophagic (human-biting) mosquitoes, and sampling will thus not be representative of vectors that are more exophagic (outdoor biting) or prefer feeding on animals (zoophagic). In other words, that sampling will be biased toward indoor biting, human host-seeking vectors. Also, each method functions differently with local vector species, their bionomics, and the local environment so validating sampling methods locally prior to widespread use is critical. For example, a method that works in one country may not work in another country based on local vector behavioral differences.

Therefore, it is important to evaluate how sampling methods function within the local context. See [Box 1](#) for a list of sampling methods included in the ESPT.

Box 1. Sampling Methods

1. Human landing catches (HLC)
2. Human baited traps (HBT)
3. Indoor resting collections (IRC)
4. CDC light trap (CDC-LT)
5. Human odor baited traps (HOBT)
6. Animal odor baited traps (AOBT)
7. Outdoor resting collections (ORC)
8. CO2 baited trap
9. Gravid traps
10. Interception traps (window exit traps (WET)/barrier screen (BS))
11. Larval surveys (LS)

These sampling methods are described further in [Annex III](#) and are referenced throughout the modules and decision trees to support the collection of minimum essential indicators.

Selecting the appropriate sampling method to answer the specific program question is equally as important. For example, if the question is, what is the vector species composition and distribution at this site to target interventions? Then human landing catches (HLCs) alone would only sample local anthropophagic vectors, and miss zoophilic vectors. The objective is to capture all vectors at the site. Similarly, if HLCs are conducted only inside and outside houses, other important sites of possible transmission will be missed, such as forest sites.

Limitations of sampling methods used and potential biases introduced in the data should be acknowledged in data analysis. For example, if indoor aspirations are conducted to capture wild female *Anopheles* to rear F1 progeny for insecticide resistance testing and to monitor the impact of IRS,

the data produced would ideally be accompanied by a note explaining that outdoor resting vectors were not factored into the analysis. Outdoor resting vectors may have very different insecticide resistance profiles. Conversely, using larval sampling to answer the same question on insecticide resistance would not specifically capture indoor resting (and thus IRS-targeted) adult mosquitoes. Larval sampling may represent a different set of vectors that may not be affected by IRS.

When using multiple sampling methods, possible interactions between methods should be factored into analysis. For example, if HLCs are combined with pyrethrum spray catches (PSCs), different houses should be used. HLC-captured samples over the night may not be present for the morning PSC sampling and vice versa. Therefore, a house sprayed with insecticide for a PSC may have lower mosquito entry which affects indoor HLCs the following night. Each sampling method may influence the other and thus affect the data collected.

Importantly, since HLCs remain the gold standard for determining human biting rate (HBR), where HLCs are not permitted, an evaluation of how an HLC-proxy sampling method (e.g., CDC light trap) corresponds to an HLC would ideally be conducted. The evaluation would compare the relative efficacy

of each method per vector species and produce a conversion factor that can be applied to the data to standardize the interpretations.⁶ These evaluations should be performed periodically (i.e., every two years based on local capacity) to capture temporal changes in vector behavior and the local environment that may affect the appropriateness of the sampling method and biases of the data.

Well-designed sampling can capture data to answer multiple questions using the same methods. For example, indoor and outdoor HLCs can be used to collect data to understand vector species compositions and human biting rate, as well as time and place of biting. Note that HLCs are not always reflective of actual exposure of humans to mosquito bites. In fact, actual exposure to mosquito bites can be more accurately determined by overlapping human behavior observations data with vector behavior data. Using one sampling method to answer multiple questions helps streamline entomological surveillance activities and optimize financial and human resources. See [Tables 8](#) and [9](#) below that describe the types of questions and entomological indicators each method may help address, along with the limitations, advantages, and disadvantages of each method.

6 Fornadel CM, Norris LC, Norris DE. Centers for Disease Control light traps for monitoring *Anopheles arabiensis* human biting rates in an area with low vector density and high insecticide-treated bed net use. *Am J Trop Med Hyg.* 2010;83(4):838–842.

Table 8. Sampling methods used to address specific types of questions and entomological indicators

	Sampling method	Mosquito behavior that the method targets	Host preference	Is the sampling method appropriate to collect data for these indicators?										Examples of traps (most common are in bold)
				Minimum essential indicator (to be selected based on the question)									Supplemental	
				Vector occurrence ^c	Vector density ^c	Larval habitat occupancy	Biting location	Biting time	Human biting rate	Indoor resting density	Insecticide resistance frequency ^f	Sporozoite rate ^g	HBI ^h	
1	Human landing catch (HLC)	Human host seeking	Human											HLC inside, HLC outside
2	Human baited trap (HBT)	Human host seeking	Human					e	e				h	Tent Trap , Ifakara Tent Trap, Furvela Trap, Odor Baited Entry Trap (OBET)
3	Indoor resting collection (IRC)	Resting behavior (indoor)	Human or animal ^a										g,h	PSC , aspiration (manual/backpack)/ Prokopack
4	CDC light trap (LT)	Human or animal host seeking	Human or animal ^a					e	e		e		e	CDC-LT
5	Human odor baited trap (HOBT)	Human host seeking	Human					e	e				h	Suna Trap
6	Animal baited trap (ABT)	Animal host seeking	Animal					e				e		Tent Trap , OBET
7	Outdoor resting collection (ORC)	Resting behavior (outdoor)	N/A										g	Aspiration (manual/backpack), Prokopack, resting pot/box, pit traps
8	CO2 baited traps	Human or animal host seeking	Human or animal ^b					e						CDC-LT with CO2 source, other traps with CO2 sources
9	Gravid traps	Oviposition seeking	N/A											
10	Interception traps	Flying, exiting, sugar or host seeking	Human or animal							(WET)				Window Exit Trap (WET), Barrier Trap
11	Larval surveys	Larvae and pupae development	N/A											Larval dipping



Yes

a. Based on location of sampling (i.e., human versus animal shelters)

b. Based on location of sampling (i.e., human versus animal shelters) and bait used

c. Using only one sampling method may bias results of vector occurrence and composition

d. Based on sampling design and location

e. Based on sampling design and method

f. IR tests using wild caught adults in the field versus F0 adults reared from wild caught larvae may produce differing results

g. Biased toward indoor resting mosquito populations

h. Biased toward human-biting (anthropophilic) mosquitoes

Table 9. Detailed sampling methods: the limitations, advantages, and disadvantages

	Sampling method	Trap name (Those in bold are more common; others are more experimental.) (Tthis is not an exhaustive list.)	Requires standard-ization (at site) ^a (Yes/No)	Condition of samples (1 = poor, 5 = excellent)	Sam-ples alive? (Yes/No)	Level of difficul-ty (1 = easy, 5 = difficult)	Capacity required ^c (low, medi-um, high)	Cost of materi-als (low, medium, high)	Which sampling method(s) can be used to determine if a different intervention may be appropriate?			Which sampling method(s) can be used to evaluate interventions currently in use?		
									LLINs	IRS	Larviciding	LLINs	IRS	Larviciding
1	Human landing catch (HLC)	HLC	Yes	5	Yes	5	High	Low	√			√ ^d	√ ^d	√ ^e
2	Human baited trap (HBT)	Tent Trap	Yes	5	Yes	3	Medium	Low				√ ^d	√ ^d	√ ^e
		ITT	Yes	5	Yes	3	Medium	Medium				√ ^d	√ ^d	√ ^e
		Furvela Trap	Yes	5	Yes	3	Medium	Low				√ ^d	√ ^d	√ ^e
		OBET	Yes	5	Yes	4	Medium	High				√ ^d	√ ^d	√ ^e
3	Indoor resting collection (IRC)	PSC	No	5	No	5	Low	Low		√		√ ^d	√ ^d	√ ^e
		Aspiration (manual/back-pack), Prokopack	No	4	Yes	3	Low	Low		√		√ ^d	√ ^d	√ ^e
4	CDC light trap	CDC-LT	Yes	3	No	2	Medium	High	√			√ ^d	√ ^d	√ ^e
5	Human odor baited trap	Suna Trap	Yes	5	Yes	4	Medium	High	√			√ ^d	√ ^d	√ ^e
6	Animal baited trap	OBET	Yes	Varies ^b	Yes	5	Low	High						√ ^e
		Tent Trap	Yes	5	Yes	3	Low	Medium						√ ^e
7	Outdoor resting collection (ORC)	Aspiration (manual/back-pack), Prokopack	No	5	Yes	3	Low	Medium						
		Resting pot/box	No	5	Yes	2	Low	Low						
		Barrier Trap	Yes	5	Yes	2	Low	Low						√ ^e
8	CO2 baited trap	Various sampling devices can be used with a source of CO2 (e.g., Tent Trap, CDC-LT, etc.)	Yes	Varies ^b	Varies ^b	Varies ^b	Varies ^b	Varies ^b	√			√ ^d	√ ^d	√ ^e
9	Gravid traps	Gravid traps	Yes	Varies ^b	Varies ^b	Varies ^b	Medium	Varies ^b			√			√ ^e
10	Interception traps	Window Exit Trap (WET)	Yes	5	Varies	4	Low	Low	√	√		√ ^d	√ ^d	√ ^e
		Barrier Screen/Trap	Yes	5	Yes	2	Low	Low				√ ^d	√ ^d	√ ^e
11	Larval sampling	Larval dipping	No	5	Yes	4	High	Low			√			^f

a. **Standardization** indicates the need to test the sampling method in an independent evaluation to examine its sensitivity and specificity when being used in a site for the first time.

b. Depends on sampling method. | c. **Capacity requirements** could include human resources (in quantity and/or skills), training, and/or equipment based on the sampling method.

d. Use these methods to look at changes in vector compositions, densities, and behaviors relative to baseline data. For IRS, the assumption is that indoor resting vector species are known to monitor trends in that particular species population. | e. Yes, use these if looking at changes in adult densities. | f. Larval sampling can be used to inform a process indicator about whether a site was treated but should not be used to evaluate the impact of the intervention.

Entomological techniques for analyzing mosquitoes

Once vectors have been sampled in the field, they are usually brought to the laboratory for analysis. Indicators including vector occurrence; sporozoite rate; frequency, intensity, and mechanism of insecticide resistance; human blood index; and bio-efficacy of insecticide, among other indicators, all require analysis with standardized entomological techniques.⁷ See [Box 2](#) for a list of techniques described in this ESPT. Most techniques require training (and retraining) and appropriate capacity. Molecular techniques (e.g., molecular species identification, sporozoite detection, etc.) require higher capacity (e.g., laboratory infrastructure, resources, advanced training, etc.). Collaboration with local or international partners may support these activities when a national malaria program capacity is limited.

Each of these techniques also has biases and similar consequences on data and analyses as the sampling methods described above. For example, the well-known morphologically indistinguishable *An. gambiae* complex has multiple species with diverse behaviors that contribute to disease transmission differently. Restricting data analysis to morphological identification only may affect the accuracy and specificity of vector species data, ultimately affecting all data and decision-making related to specific vector species, including insecticide resistance.

Box 2. Entomological Techniques³

1. *Anopheles* identification keys
2. Molecular identification – PCR
3. Salivary gland dissections
4. Ovary dissections
5. CS ELISA – sporozoite detection
6. BM ELISA – host blood detection
7. PCR – parasite detection
8. WHO tube assay
9. CDC bottle assay
10. Kdr PCR or biochemical assay
11. Cone bioassay

7 Doolan DL (Ed). (2002) *Malaria Methods and Protocols*. Humana Press; 2002.

These entomological techniques are described further in [Annex III](#) and are referenced throughout the modules and decision trees to support the collection of minimum essential indicators.

Methods for assessing human behavior and high-risk populations

To appropriately and efficiently target vector control interventions, it is important to know which humans to target and *when* and *where* to target humans who are exposed to mosquito bites. Data from surveys on human behavior and high risk populations (HRPs) analyzed together with data on vector bionomics and intervention efficacy may help determine gaps in protection and local drivers of transmission, including drivers of residual transmission. While there is a growing research agenda on this topic,^{8,9} program-oriented methods are currently available for national malaria programs to consider using (see [Box 3](#)).

Throughout the subsequent modules, we include these methods for integration with entomological surveillance activities for programs with the resources to employ them.

As described in *A Malaria Elimination Guide to Targeted Surveillance and Response in High Risk Populations* (UCSF 2017), malaria HRPs are groups of people who share socio-demographic, geographic, and/or behavioral characteristics that place them at increased risk of infection. These populations are often characterized as having poor access to or low utilization of health services and interventions,¹⁰ or behaviors associated with increased exposure to *Anopheles* mosquitoes including those related to occupation, (e.g., farming, forest, and mining-related work.)¹¹ Identifying and understanding specific characteristics of populations at risk for malaria, and where and when they come into contact with vectors, enables national malaria programs to better tailor and target interventions.

8 Monroe A, Mihayo K, Okumu F, et al. Human behaviours and residual malaria transmission in Zanzibar: findings from in-depth interviews and direct observation of community events. *Malar J*. 2019;18 (220).

9 Edwards HM, Chinh VD, Duy BL, et al. Characterising residual malaria transmission in forested areas with low coverage of core vector control in central Viet Nam. *Parasit Vectors*. 2019;12: 454.

10 Chen I, Thanh HNT, Lover A, et al. Malaria risk factors and care-seeking behaviour within the private sector among high-risk populations in Vietnam: a qualitative study. *Malar J*. 2017;16 (414).

11 Jacobson JO, Cueto C, Smith JL, et al. Surveillance and response for high-risk populations: what can malaria elimination programmes learn from the experience of HIV?. *Malar J*. 2017;16 (33).

Box 3. Methods for Assessing Human Behavior and High Risk Populations

Any data captured using example methods below should be analyzed with entomological, intervention, and epidemiological data, including data from passive and active case detection and case investigations, as available. Collectively, this data can provide important evidence about potential gaps in protection that may allow ongoing transmission, among other things.

User experience with, and acceptance of, vector control interventions also help to explain human behavior and the use or non-use of interventions. Acceptability data should be collected when feasible and incorporated into the analysis of an existing vector control strategy.

Example of human behavior survey methods

Objective: to understand how human behavior overlaps with vector behavior to identify primary points of human-vector contact for intervention targeting

- Collate data from case investigation forms (as available), including travel history, occupation, use of preventive interventions, and other data that could provide insight into the case's behavior and activities potentially leading to an increased risk for malaria infection.
- Conduct Human Behavior Observations (HBOs) during HLCs to document time and duration that humans spent outdoors versus indoors and under ITNs and/or in a sprayed house (see [Module 7](#) for examples of how HBOs can be incorporated into entomological surveillance and [Annex IV](#) for an example HBO data collection form).
- Conduct surveys on time and duration that humans spent outdoors versus indoors or in higher risk areas through self-administered (less optimal) or staff-administered (more optimal) questionnaires and/or daily activity logs maintained by community members.
- Develop seasonal calendars with community members with information on timing of peaks of disease, when people move (e.g., religious festivals, cattle-related movement), main agricultural activities (e.g., planting, harvesting, or movement of livestock), and whether these activities

may include outdoor work during vector biting times.¹²

- Conduct participatory mapping with village chiefs, religious leaders, and community groups to help map where people live, their patterns of movement, location of health services, land use, vegetation and water bodies, etc. Mapping also supports community engagement in local vector surveillance and control.

Example high-risk population (HRP) survey methods

Objective: to identify and characterize HRPs driving transmission and specific behaviors and intervention gaps within these populations to improve targeting of entomological surveillance and vector control response activities

- Conduct a thorough review of existing epidemiological surveillance data. Extract meaningful case information such as demographics (e.g., age and gender), occupation, seasonality, clustering, etc.
- Collate data from case investigation forms (as available) and health facility data to understand the distribution of cases and identify patterns, including whether cases seem to be clustering geographically or by other possible risk factors, i.e., travel history, occupation, etc.
- To plan for tailored and targeted surveillance, conduct a formative assessment (qualitative research) to gather, update, review, and analyze current knowledge of HRPs, including travel and work patterns, social network connectivity, night-time activities, sleeping patterns, and other behavioral risk factors, and intervention gaps, that will help to optimize implementation of interventions.¹³ Geolocate (i.e., map) work sites where people spend time that might put them at higher malaria risk, as well

12 WHO. A toolkit for integrated vector management in sub-Saharan Africa. World Health Organization, Global Malaria Programme, Geneva. 2016.

13 Smith JL, Auala J, Haindongo E, et al. Malaria risk in young male travellers but local transmission persists: a case-control study in low transmission Namibia. *Malar J.* 2017;16 (70).

as outdoor or semi-outdoor venues where HRPs frequent or congregate. This will help determine if venue-based interventions would be appropriate (also for time-location sampling and SB-RACD, see below). Communities will often have the best knowledge of possible HRPs and their activities, so collaborations with communities and community groups is key.

- To characterize malaria HRPs and identify context-specific risk factors for malaria that could then be targeted by malaria prevention interventions, a case-control study can be implemented through questionnaires administered to malaria cases and a comparison group of controls identified at health facilities.^{13,14}
- To monitor malaria transmission and interventions among HRPs, targeted sampling such as time-location sampling can be used to access and survey people at specific venues and times where HRPs are more likely to be present (i.e., forest worksites or border crossing points). This provides an ongoing assessment of infection prevalence among HRPs at these sites can be conducted along with other key indicators, such as intervention use and associated risk behaviors. If there are established times and places where HRPs gather, interventions could be provided at these locations (e.g., distribution of LLINs).

14 Grigg MJ, Cox J, William T, et al. Individual-level factors associated with the risk of acquiring human *Plasmodium knowlesi* malaria in Malaysia: a case control study. *Lancet Planet Health*. 2017;9 (3).

- To improve routine surveillance, socio-behavioral reactive case detection (SB-RACD) incorporates targeted screening of HRPs at specific sites and through social contacts, based on a common set of risk criteria with an index case. This approach is especially useful in contexts where transmission occurs away from home (e.g., the forest and forest-fringe).^{9,15} As part of routine surveillance based on a set of risk criteria, SB-RACD involves screening at specific venues or work sites^{16,17} and the social contacts of malaria index cases that recently shared work or other locations.

Evidence on human behavior and HRPs, combined with local evidence on vectors, can inform a more targeted and tailored vector control strategy.

For more guidance on these HRP methods, see the Malaria Elimination Guide to Targeted Surveillance and Response in High Risk Populations by the UCSF Malaria Elimination Initiative: shrinkingthemalariamap.org/tools/high-risk-populations-surveillance-and-response-guide.

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- 15 Herdiana H, Cotter C, Coutrier FN, et al. Malaria risk factor assessment using active and passive surveillance data from Aceh Besar, Indonesia, a low endemic, malaria elimination setting with *Plasmodium knowlesi*, *Plasmodium vivax*, and *Plasmodium falciparum*. *Malar J*. 2016;15 (468).
- 16 Jacobson JO, Smith JL, Cueto C, et al. Assessing malaria risk at night-time venues in a low-transmission setting: a time-location sampling study in Zambezi, Namibia. *Malar J*. 2019;18 (179).
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Module 4. Select Sites and Survey Type

The selection of sites for entomological surveillance should reflect the heterogeneity of malaria transmission in the country and account for geographic variation in malaria epidemiology, importation risk, and receptivity (see Glossary in [Annex V](#) for definitions).¹⁸ There are three types of sites described in the ESPT:

- **Sentinel site:** fixed sites that represent different ecological and epidemiological regions of a country, including areas with high receptivity and importation risk, as well as areas with risk of reestablishment where malaria transmission has been interrupted (if resources are available). Entomological surveillance based at sentinel sites is important for measuring trends over time.
- **Focus:** a defined, circumscribed area situated in a current or former malarious area that contains the epidemiological and ecological factors necessary for malaria transmission.¹⁹ In practice, a focus is often a village or small clusters of neighboring villages. Entomological surveillance in foci is important for informing the most effective response to reduce and interrupt transmission.
- **Targeted site:** a site targeted for a spot survey to answer a specific question or set of questions. A targeted site could include an area experiencing an outbreak or an increase in importation risk or receptivity.

Program capacity and available resources will always limit the scope and scale of entomological surveillance activities. With limited capacity and resources, the priority must be to concentrate entomological activities in areas with higher malaria transmission relative to the rest of the country. This is especially helpful in higher burden countries, or in specific regions to answer a specific programmatic question. In low transmission countries nearing elimination, however, priority areas should include those with high importation risk and/or receptivity to support prevention of reestablishment.

When resources are available, scope of activities and scale of implementation can expand as long as the data generated is for decision-making. *Quality* of data should be prioritized over *quantity* of data. [Figure 3](#) describes the process for site selection.

Programmatic questions can be answered at sentinel sites, during foci investigations, and through spot surveys at targeted sites depending on the question and the geographic scale of interest.

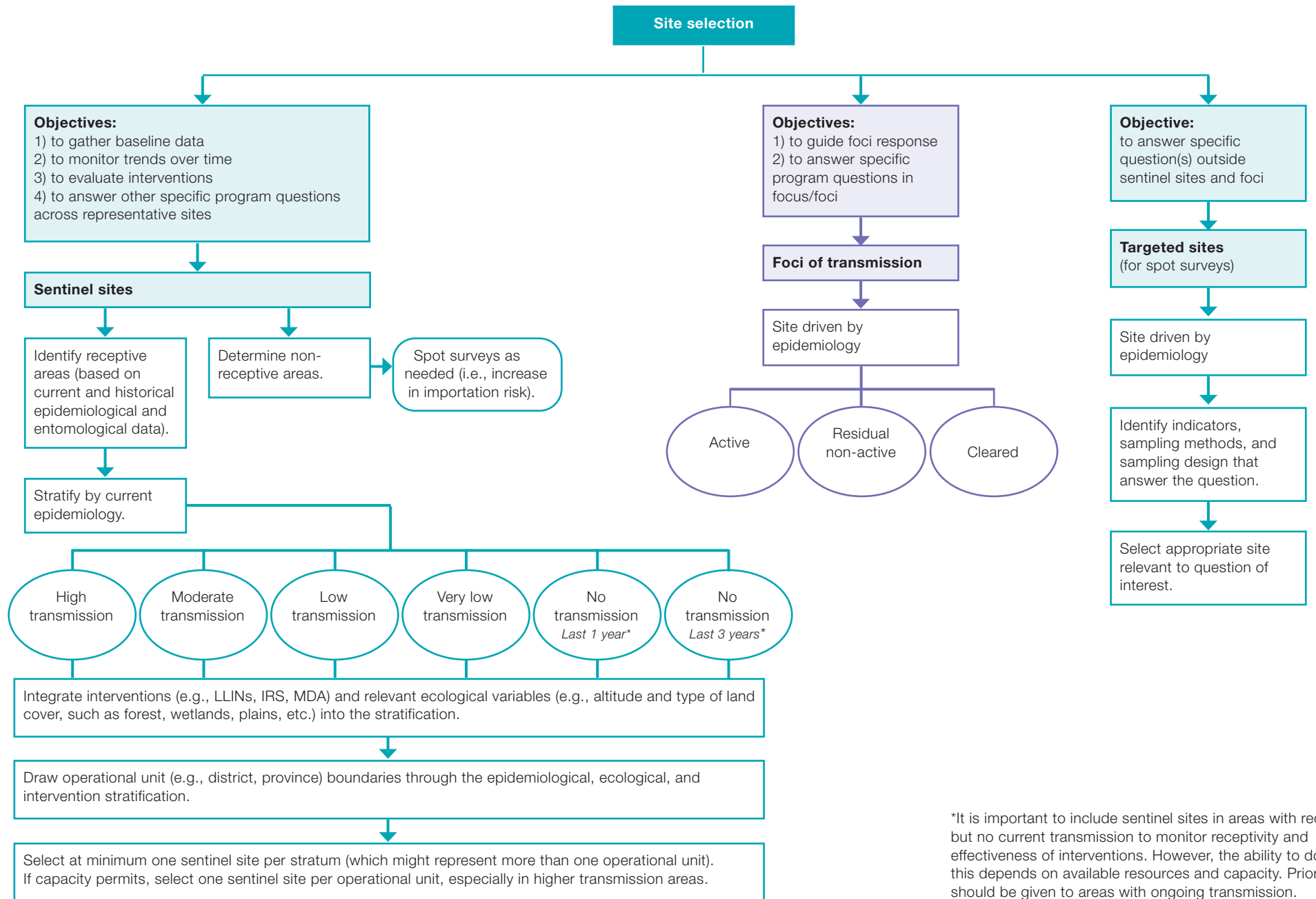
Types of surveys by type of site

The ESPT covers four types of entomological surveys: baseline, routine, foci, and spot surveys. Below is guidance on the rationale for each survey, the type of site for each survey, and the minimum frequency of data collection within and across years. Frequency ultimately depends on the capacity and needs of each malaria program.

18 WHO (2018) Malaria Surveillance, Monitoring & Evaluation: a reference manual. Chapter 5: Entomological surveillance and response. World Health Organization. Geneva.

19 WHO (2016) WHO malaria terminology. World Health Organization, Geneva.

Figure 3. Determining sites for entomological surveillance activities



*It is important to include sentinel sites in areas with recent but no current transmission to monitor receptivity and effectiveness of interventions. However, the ability to do this depends on available resources and capacity. Priority should be given to areas with ongoing transmission.

Table 10. Sampling site and frequency by survey type

Type of survey	Trigger for survey	Type of site for survey (sentinel, focus, targeted)	Minimum frequency of data collection within one year	Minimum frequency across years
Baseline survey	Lack of data for multiple years	Sentinel, focus	One sampling period per site per season* over one year	Repeated every ~3 years (based on capacity) and/or when epidemiology, importation risk, and/or receptivity change significantly and/or when a new vector control intervention is under consideration
Routine survey	Continuous	Sentinel	One sampling period per site per season* over one year	Repeated every year
Foci investigation	Index case	Focus	One sampling period per focus per season over one year	Repeated in active foci every year, triggered by the first few index cases of the malaria season
Spot survey	As needed to answer a specific question	Targeted site	Depends on the sampling method to answering the question	As needed

*Data collection during peak transmission season(s) is the priority; however, non-peak transmission seasons might have very different transmission dynamics and drivers that would ideally be captured as well.

Sentinel sites

Looking at Figure 3, how is “receptive” vs. “non-receptive” defined?

For the purposes of this tool, a receptive area is based on suitable temperature, humidity, and altitude for vector survival, humans present, and one of the two indicators:

- Adult vector occurrence (yes/no)
- Immature vector occurrence (yes/no)

Data from the last three years should be reviewed to help determine receptivity of an area. Note that though some countries use adult vector density to describe the “level” of receptivity i.e., high or low, this density does not always correlate to risk of transmission. Low transmission areas may experience significant malaria outbreaks with low vector density.

What exactly is a “site”?

A sentinel site may be one village or cluster of neighboring villages. The site should consist of a sufficient number of households or potential larval habitats for the sampling design (see [Module 5](#)). The site should be relatively accessible for the entomological surveillance team. In some countries, sentinel sites are only for field collections. In other countries, sentinel sites also have a basic entomological laboratory and/or insectary for sample processing, morphological identification, insecticide resistance testing, and/or data entry. The definition of a “site” may vary based on the country and the question. Programs should determine their definition of a site, apply the definition countrywide, and remain consistent. Sites should be consistent with the sampling design ([Module 5](#)). For example, a “site” could include two distinct villages based on the sampling design and to ensure standardized sampling procedures.

Why use sentinel sites?

- For **baseline surveys** to gather baseline data on local vector bionomics for planning vector control interventions ([Module 7](#)).
- For **routine surveys** to monitor trends over time of priority indicators that will inform changes to vector control strategy ([Module 8](#)).
- To **answer specific program questions** across established representative sites, often with available historical data for reference.

How many sentinel sites are needed?

- Country stratification should guide initial sentinel site considerations. At a minimum, there should be one sentinel site per stratum in a country's stratification.
- [Figure 3](#) provides guidance on how to select sentinel sites based on receptivity, epidemiology, presence of interventions, and ecological zones. It is likely that country stratification is already based on these variables. Adding administrative boundaries (i.e., province) within the eco-epidemiological stratification is useful for planning and budgeting purposes.
- In very low transmission countries where transmission may be limited to a few areas, micro-stratification should be done in those areas and sentinel sites placed in those strata as feasible. In this case, microstratification should include urban/peri-urban vs. rural, accessibility, and local ecology (e.g., coastal vs. forest).
- PMI guidance states that at least two sites for insecticide resistance monitoring should be identified in each administrative division where PMI supports monitoring. An administrative division is the smallest unit in which a change in vector control policy can be applied. This is typically a state, province, region, or county for LLINs and districts for IRS. A site may consist of several villages in close proximity.²⁰
- Available resources and capacity will ultimately determine the number of sentinel sites irrespective of transmission level. If a program is deciding between quantity of sites versus quality of those sites (including the activities performed and data generated), quality should always be the priority (i.e., avoid stretching resources too thin, which could lead to inconclusive data). A program may also decide to use targeted sites rather than sentinel sites based on available resources and the question the program is trying to answer.

Determining how many sites are enough sites while maintaining robust data quality is challenging. Consider the data generated through entomological collections at the selected sentinel sites in light of the program's priority questions: are the data conclusive, and can the program make evidence-based decisions with the data? If the answer is yes, then it is possible the program has attained a sufficient number of sentinel sites. On the other hand, if the data is insufficient or inconclusive, then consider the following:

- » Is data being improperly processed/managed due to insufficient capacity? If so, focus should be on improving data management and interpretation.
- » Are data management and analysis capacities present, but insufficient data is being collected? If so, perhaps increasing the number of sites would be useful. Be sure site selection is based on up-to-date stratification ([Figure 3](#)) and the program question(s).

When should I consider increasing/decreasing/moving sentinel sites?

- Ongoing monitoring in the same sites is useful to assess trends over time, as long as that data is answering program questions and being used for decision-making.
- When malaria programs update their stratification or intervention strategy, sentinel sites should be reassessed to make sure they are still representative of strata and the key program questions. In other words, while maintaining historical sites for longitudinal monitoring may be important, sites must continue to have relevance for the country's current malaria transmission landscape and must be generating data that directly informs program decision-making. If sites do not meet those criteria, programs should consider updating the placement of their sites.
- Any increase to the number of sites should be based on available resources, capacity, and ability to maintain quality control in existing sites. The program should also consider whether time-limited spot surveys in targeted site(s) might be more appropriate to answer a specific question(s) as an alternative to establishing a new site. This might also be a more cost-effective option.
- A decrease in the number of sites may be necessary to maintain high quality data with the available resources and capacity. Programs may choose to prioritize sites in areas of higher transmission, reducing sites in areas of low or no

20 US President's Malaria Initiative. FY 2020 Technical Guidance. 2019.

transmission. As mentioned above, when countries are nearing elimination, it becomes important to maintain sites in areas of low or interrupted transmission to monitor receptivity. If malaria has been eliminated from a particular area, the program will need to determine whether or not to maintain the sentinel site in that region depending on the availability of resources.

Are there other variables that should help determine placement of sentinel sites?

Additional data can help inform placement of sentinel sites, including:

- Demographics, including human population, settlement pattern, and variables related to importation risk (i.e., population movement, major economic and development activities, and cultural and socio-political aspects).
- Drug and/or insecticide resistance.
- Entomology, including vector species and behavior, presence and location of permanent and temporary larval habitats, and agricultural production, among other data. In fact, sentinel site placement could be based on data gathered through a baseline survey.
- Land use, including large construction projects, agricultural areas, and deforestation.
- Access to diagnosis and treatment.

Foci

Entomological surveillance as part of foci investigation and response is most relevant for low and very low transmission areas where programs have a foci classification and management system. In this case, entomological activities in foci should be triggered by epidemiology.

The WHO defines three types of foci:²¹

- Active: focus with ongoing transmission
- Residual non-active: focus where transmission was interrupted recently (1–3 years)
- Cleared: focus with no local transmission for >3 years

As noted above, in practice, a focus is often a village or small cluster of neighboring villages. In some countries, a focus might be a health facility

catchment area. Entomological surveillance in foci is important for informing foci response to reduce and interrupt transmission.

In an active focus, entomological investigations may be similar to a baseline or routine survey but only in a focus, not at a sentinel site. However, the scope of activities should be limited to the minimum required to inform an effective focus response. This is especially important in areas with limited resources but with many active foci.

Foci investigation often includes reactive case detection (RACD), or testing household and community members for malaria within a circumscribed area around an index case(s) for malaria. Therefore, it's also likely that the individuals involved in foci investigations (e.g., surveillance officers and health workers) are different from the individuals involved in sentinel site-based surveillance (e.g., trained entomology technicians), which may affect the scope and scale of foci investigations.

In residual non-active and cleared foci, entomological investigations would be triggered following diagnosis, treatment, and investigation of an index case. The objective of the entomological investigation in this case would be to inform a rapid response to immediately interrupt any possible onward transmission.

Recent data from nearby representative sentinel sites can be applied to foci, especially in a very resource constrained environment. Further guidance on foci investigation is in [Module 9](#).

Targeted sites

Targeted sites are sites selected based on a specific question for a spot survey. In this case, sites can be any geographic area. For example, a target site might be a district that is experiencing a malaria outbreak, and the program wants to understand the drivers of the outbreak. Or there are changes in importation risk (e.g., a new group of migrants from a malaria endemic region or country) or receptivity (e.g., a new construction site) that triggers a spot survey to identify present vectors to assess risk of malaria transmission in that area.

Reference [Module 3](#) for sampling methods to ensure representative sampling of the targeted site using a spot survey to answer the question appropriately.

21 WHO. Malaria Surveillance, monitoring & evaluation: a reference manual. World Health Organization. Geneva. 2018.

Module 5. Design Sampling for Operational Purposes

Before developing a sampling design, the program should identify the priority question(s) and/or decisions that need to be made, as well as the corresponding indicators relevant to address the question(s). The specific question(s) will determine the sampling plan to collect the data required to measure the selected indicators. Below is a step-by-step guide to working through the key aspects of sampling design development for an entomological investigation.

Step 1. Determine the sampling site

A sampling site is the collection locality (geography) from where mosquito samples are collected to obtain relevant data to measure the indicators selected. As described in [Module 4](#), these sites can be sentinel sites for baseline or routine surveillance, foci of transmission, or another area(s) of interest where a spot survey may be needed to answer a specific question. The sampling site(s) will vary according to the program's question (see [Table 11](#)).

Limited available human capacity, financial resources, and accessibility may constrict the size and number of the sampling sites. If downsizing is necessary, then circle back to the primary question being asked to ensure that the sampling site(s) selected is relevant to the question posed. It is also essential that caveats and limitations to final site selection be noted, recorded, and reported.

Table 11. Example questions posed with corresponding appropriate sampling site

Program question	Sampling site(s)
Where are the villagers of Village X exposed to <i>Anopheles</i> mosquitoes?	Village X + other areas where villagers are present during <i>Anopheles</i> biting times (e.g., village X + surrounding forest worksites)
Health Facility A and B are reporting abnormally high number of malaria cases. What are the entomological drivers of this outbreak?	Catchment areas of Health Facility A and B
Is there presence or absence of insecticide resistance to the active ingredient used for IRS and/or LLINs in Region Y?	All sentinel sites in Region Y where the intervention was deployed

Step 2. Determine the sampling unit

The sampling unit is an individual unit for mosquito collection within sampling sites. The sampling unit can be a village, a house, a cattle shed, a forest or farm worksite, or a water body, for example. The question of focus and indicators will indicate what criteria should be applied to select the appropriate sampling unit ([Table 12](#)). The sampling unit must be standardized across all selected sampling sites to collect data that is comparable and so that units can be analyzed together, across collection sites.

Table 12. Example questions with corresponding possible sampling unit selection criteria

Program question	Indicator	Sampling unit	Possible sampling unit selection criteria
How is IRS affecting the indoor resting density of <i>Anopheles</i> in Village X?	Indoor Resting Density	Houses*	<ul style="list-style-type: none"> Sprayed houses. Samples of all wall types present (mud, concrete, zinc, etc). Inhabited houses—people sleeping inside every night
What is the human biting location of <i>Anopheles</i> in Village X?	Human Biting Rates	Houses* and other structures in village	<ul style="list-style-type: none"> Inhabited houses (inside and outside) Spaces where people are present during <i>Anopheles</i> biting periods such as outdoor cooking shelters

*Note: based on the question being posed around the resting behavior of local *Anopheles*, cattle sheds and other relevant structures could be included here.

Step 3. Allocate the sampling units

The allocation of sampling units is the selection of sampling units in the sampling sites that are to be included in the entomological investigation. For example, if inhabited houses in Village X are the sampling units, then a decision has to be made about which subset of houses from Village X will be included in your investigation. For sampling unit allocation, work through the four following points:

1. Does historical data relevant to the question already exist for the sampling site selected?
 - a. If yes, use the historical data to help guide the allocation of sampling units (see [Example Case 1](#)).
 - b. If no, then random allocation of sampling units is appropriate. Note that random allocation of sampling units can either be completely random (i.e., not using any knowledge or criteria to guide random selection) (see [Example Case 2](#)), or it can be random within a set of criteria within the sampling site (see [Example Case 3](#)). The same sampling unit allocation must be applied across all sampling sites in order to maintain standardization, and thus, comparability of data across sites.
2. How many sampling units (i.e., sample size or number of replicates) should be allocated within the sampling site(s)?

The sample size or number of replicates required to correctly address the question of focus is dependent on the question as well as available human and financial resources. Biostatisticians determine ideal sample sizes through complex statistical power calculations. Often times, resource limitations will not allow for a large enough sample size to attain statistical power. However, especially for operational purposes, this limitation should not always halt an entomological investigation. Iterations of sample design should occur until a feasible plan that can yield informative data to answer the question while accounting for capacity constraints is formulated. Thus, criteria for determining a meaningful and feasible sample size are entirely context-specific.

Data that is generated from a survey that cannot achieve statistical power has the potential to still be informative and relevant for a program. Thus, the sample size required should be in alignment with what is feasible given available human and financial capacities, while maintaining scientific rigor.

Selecting the same number of sampling units in each sampling site will make the sampling data more standardized, and therefore more straightforward to compare across sites. However, program capacity across sites may vary, and hence, unequal counts of sampling units may be selected across the sampling sites. This is acceptable, so long as differences in sample sizes are recorded, reported, and correctly accounted for in the data analysis.

Example Case 1. Using historical data to guide allocation of sampling units

Question: What are the primary and secondary vectors in Village X?

Sampling site: Village X

Sampling unit(s): Structures, including houses, animal enclosures, outdoor or semi-outdoor gathering areas (e.g., cooking shelters)

Historical data: Pre-existing relevant data in Village X suggest higher density of vectors in lower lying areas of Village X compared to areas of elevation

Sampling unit allocation: Given the historical data, two-thirds of sampling units are allocated to areas of lower elevation and one-third of sampling units are allocated to areas of higher elevation

Example Case 2. Absence of historical data: application of random allocation of sampling units without any criteria

Question: What are the primary and secondary vectors in Village X?

Sampling site: Village X

Sampling unit: Structures, including houses, animal enclosures, outdoor or semi-outdoor gathering areas (e.g., cooking shelters)

Historical data: None

Sampling unit allocation: Use a list of Village X structures and a random number generator to select a set number of sampling units (structures)

Example Case 3. Application of random allocation of sampling units based on relevant criteria

Question: What are the primary and secondary vectors in Village X?

Sampling site: Village X

Sampling unit: Structures, including houses, animal enclosures, outdoor or semi-outdoor gathering areas (e.g., cooking shelters).

Historical data: None

Sampling unit allocation: Use a list of Village X structures and separate into groups based on structure type. Select a random set within each group, for example, a random set of human dwellings and cow sheds

In addition to frequency over the year, programs should consider frequency within each sampling period (e.g., sampling three times per year and for a duration of five days during each of the three sampling periods). Again, there is no rule for the frequency within each sampling period; more days/nights will likely produce more data, but it depends on available capacity and again, quality data over quantity should be the priority.

Timing of sampling is closely linked to the frequency of sampling. Appropriate timing of sampling is essential to collecting informative data (see [Example Case 4](#)). Questions that are related to evaluating the impact of vector control tools should consider mode of action of the intervention, mosquito life stage targeted by intervention, and timing of program roll-out of interventions across sampling sites. Baseline surveys should seek to time collections at various points throughout the transmission seasons to account for variation in vector bionomics throughout the seasons.

Step 5. Determine the sampling method

The sampling method used may have the biggest impact on the data and whether the question has been answered appropriately. Careful consideration of exactly what each sampling method measures is necessary in choosing a suitable sampling method as described in [Module 3](#). Standardization and optimization of the sampling method is vital. Ideally, individuals conducting the sampling should be trained in an identical manner with the aim to produce near-identical sampling. Variances from the standard procedures should be documented. As described in [Module 6](#) below, written records should be kept by date, location, and person sampling, with a brief description of procedures used.

Step 6. Set the frequency of sampling

The frequency of sampling is dependent on the question and on available human and financial resources. [Table 10](#) in [Module 4](#) describes minimum frequency for different types of surveys (e.g., baseline, routine, focus investigation, and spot survey). Capturing different seasons (wet vs. dry) and before, during, and after malaria transmission seasons is especially important to look at temporal trends among vector populations. More frequent sampling can often produce more representative data; however, quality of data should always be prioritized over quantity.

Example Case 4. Timing and frequency of sampling

Question: What is the residual efficacy of a new insecticide being used for IRS?

Timing and frequency of sampling:

- If resources permit, Option 1: sampling begins immediately following spraying, and subsequently occurs once per month until *Anopheles* mortality is below 80%.
- If resources are limited, then Option 2: sampling begins immediately following spraying, and subsequently occurs once every 2 months following spraying until (or beyond?) 6 months following spraying, or until *Anopheles* mortality is below 80%.

Module 6. Manage Entomological Data

The following section assumes that the malaria program has

1. Formulated its priority question(s), and
2. Established the indicators, sampling methods, sites, sampling design, and entomological techniques that will be appropriate to address the questions of focus.

Example A (field):

1. Question: When and where do *Anopheles gambiae* bite humans in Site X during peak malaria season?
2. Sampling design: Conduct five nights of HLCs inside and outside four houses during peak malaria season.

Example B (lab):

1. Question: Are *Anopheles gambiae* from Site X resistant or susceptible to pyrethroids?
2. Laboratory methodology: Conduct WHO Tube Tests with wild-caught larvae reared to adults; use females only if numbers permit, and use susceptible females as control.

Entomological data collection

In preparation for entomological data collection in the field and in the lab, the appropriate entomological field/lab data collection forms must be formulated. The field/lab data collection forms ensure that the data collected in the field or in the lab is relevant to the questions investigated.

Step 1: Identify which data collection forms are required

For each activity, there must be an associated entomological data collection form. This is often a paper form, but some programs prefer to record data directly on electronic tablets.

- **For Example A**, a field form to collect data during the HLC collections must be implemented.
- **For Example B**, a lab form to collect data during the insecticide resistance testing procedure must be implemented.

Step 2: Identify pre-existing field entomological forms related to the program's questions of focus and adapt as required based on indicators selected

Pre-formulated entomological data collection forms are compiled in both WHO and CDC manuals. These forms are excellent examples to indicate the minimum data points that must be recorded for common entomological field and lab activities (e.g., WHO Tube Test, CDC Bottle Bioassay). In some instances, such forms are adequate as written to address certain programmatic questions. However, in other instances, these forms might not consider all the data points required to address other programmatic entomological questions. In this case, the pre-existing form may serve as a template that can be modified accordingly, and tailored to the program's specific question(s), such that all the required data points are considered by the modified form.

For example, in [Example A](#), the program may want to adapt an HLC form to include a column where the collector indicates hour-by-hour whether or not rainfall occurred in order to observe whether or not absence/presence of rainfall is correlated with increases or decreases of collected *Anopheles* during the collection night. In [Example B](#), the corresponding lab form would need to include two columns per replicate to record the numbers of both females and males tested; this is because males may be included in the bioassays if insufficient numbers of females were obtained from the larval collections.

Step 3: Establish data dictionaries

Each entomological lab/field data collection form is comprised of specific column headers to ensure that the appropriate data is collected in a standardized manner. Moreover, such forms are likely used by more than one person. Thus, it is crucial that every user of these forms have access to and abides to a corresponding data dictionary.

The data dictionary includes the description of each column header, as well as the notation. For [Example A](#), below is an excerpt from the data dictionary corresponding to the addition in the HLC form.

Column Header	Description	Notation
Duration of Rainfall	Provide the length of time during which rainfall occurred each hour.	<ul style="list-style-type: none"> Indicate the duration in minutes (e.g., if it rained for 1 hour, write: 60) If no rainfall occurred, write: 0

The data dictionary should be included at the back of each field and lab collection form.

Data management

Data entry and cleaning

The entomological data collected in the field and/or in the lab must be entered into an electronic version of the form to enable further data processing and subsequent data analyses with statistical methods. Thus, each entomological field/lab data form should have its corresponding electronic form. Such electronic forms can be formulated in data entry programs such as Access.

Once the data has been entered, the data must be reviewed and cleaned in preparation for the analysis. Data cleaning involves turning all data entries into entries that can be used for analysis. This will depend on the platform used for data entry and analysis (e.g., Excel, R Studio, etc.). The three points below highlight the key aspects to consider during the data cleaning process.

- **Empty cells.** No cells should ever be left empty. If the cell is empty, determine if this is because the person entering the data simply forgot to enter the particular data point, or if it is because no data was inputted by the data collector. Ideally, quality control would be conducted in the field to ensure accurate and complete forms by the data collectors. Be sure to indicate the absence of data collected in every instance.

- **All formatting is standardized.** Ensure all data points are entered in the same format across all data entries. For example, if the format to enter the collection date is DDMMYYYY, then all dates must be entered in this format.
- **Quality check.** Data cleaning is another opportunity to verify the quality of the data entry that has been completed. Verify that the data entered is correct, for example, by entering the data a second time and cross-checking. During data entry verification, it is likely that you will come across errors, and end up having to verify the data entry multiple times. If you see any data entries that seem odd or incorrectly entered, be sure to verify these data points. You can verify data entry by simply going back to the corresponding original paper forms filled out by the data collector. Or, you can randomly select 10 paper forms to verify the quality of the data entry.

Data storage

The electronic forms should be securely stored in a database. The ability to input data into this database should be restricted to individuals who have been trained to properly enter the data. Historical entomological data is important to keep and to maintain accessibility, and thus, the database should enable the accumulated storage of yearly entomological data. Paper forms of the corresponding electronic forms should be kept for at least one year, or at least until data quality checks and analysis are completed. After these activities are complete and no further reviews are necessary, the paper forms may be discarded, as the data should remain recorded in the database.

Be sure to keep multiple back-up copies of the database.

Module 7. Decision Trees by Indicator and for Baseline Surveys

Below are a number of decision trees by the following indicators or indicator groupings:

- Baseline A. Vector occurrence and density
- Baseline B. Vector biting behavior
- Baseline C. Indoor resting density
- Baseline D. Host preference
- Baseline E. Insecticide resistance
- Baseline F. Intervention efficacy
- Baseline G. Larval habitat occupancy

These decision trees can be used for:

1. Baseline surveys at sentinel sites to help characterize transmission, inform intervention selection and deployment, and evaluate existing interventions
2. Baseline surveys in foci to help characterize transmission and inform foci response
3. Spot surveys to answer specific questions, especially in areas of outbreaks or plateauing transmission

The decision trees guide the user through data collection and data interpretation to inform a

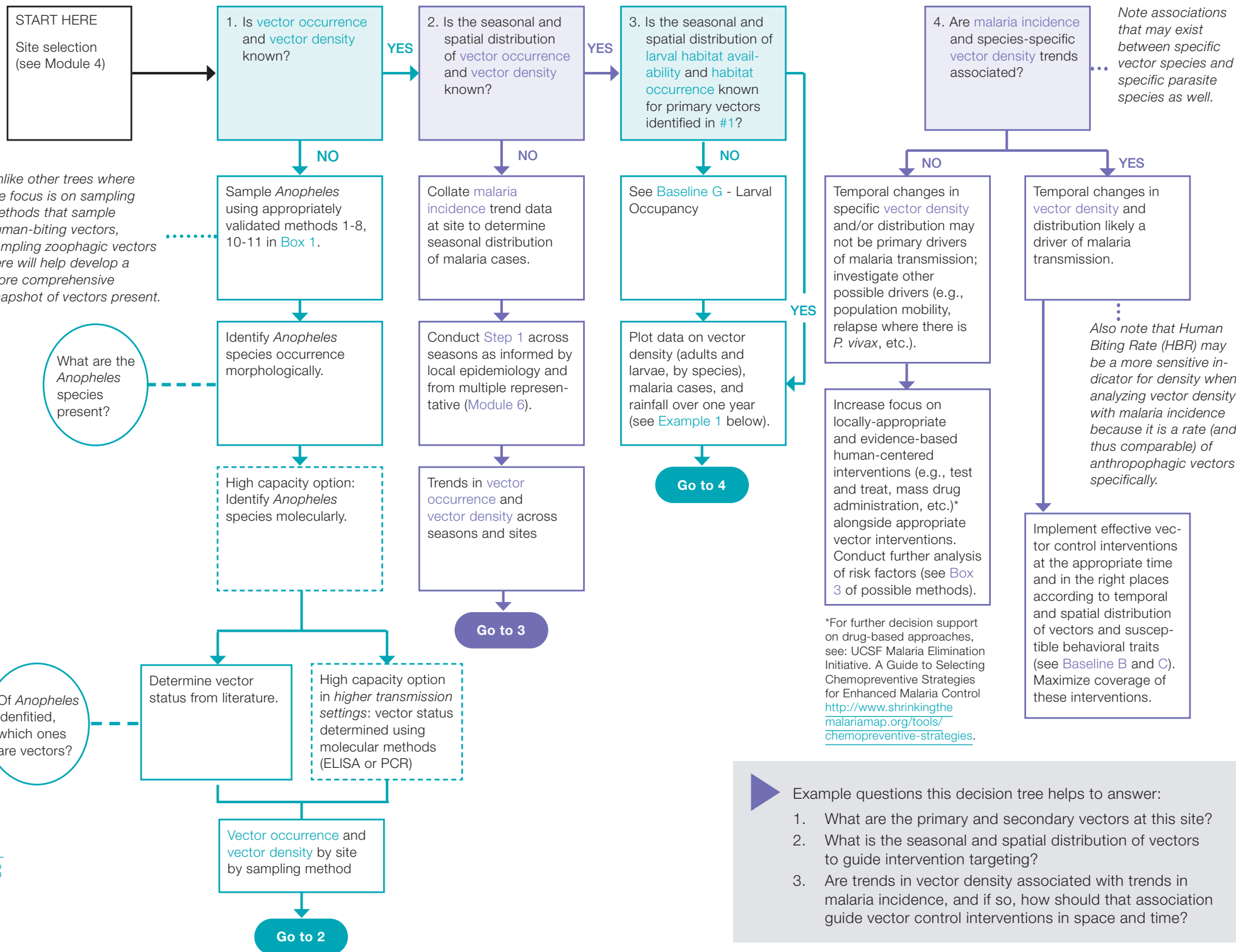
program decision about vector control or other interventions. They are especially useful in calling attention to gaps in protection that may exist leading to onward transmission. They also highlight where epidemiological, rainfall, and other data should be integrated into analysis.

Each step of the decision trees first asks, is [indicator] known at this site? In the context of this ESPT, “known” means that data has been collected recently i.e., within the last year. If the answer is yes, then the user should move to the next step (to the right) within the decision tree.

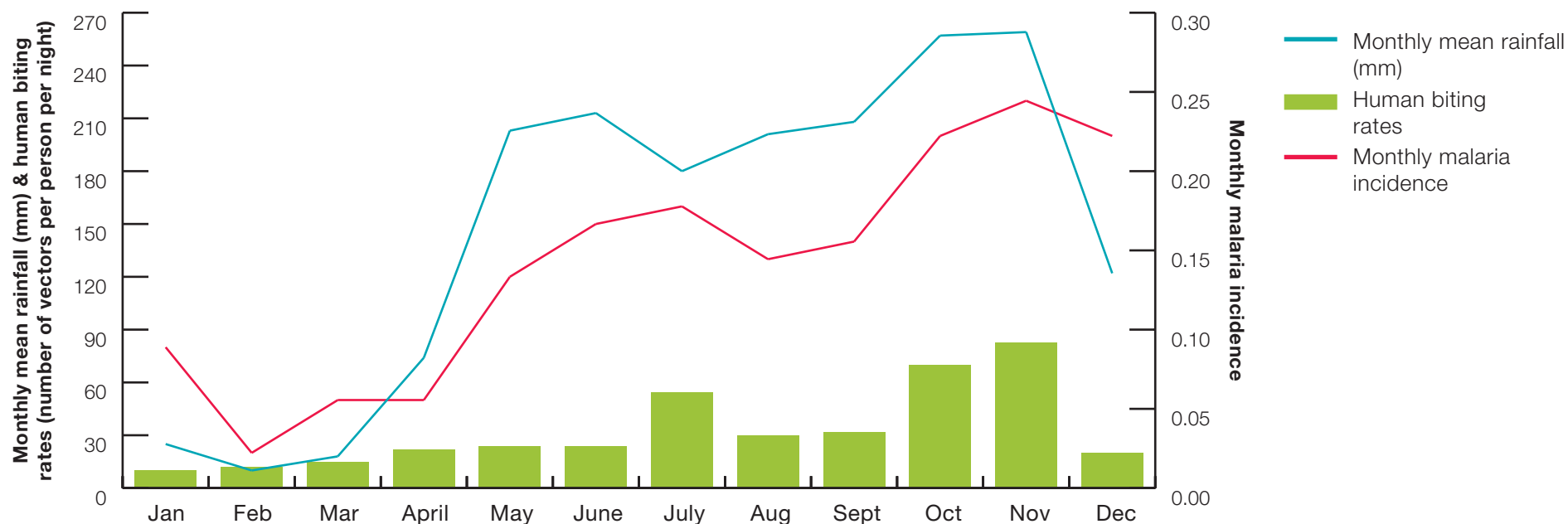
All steps in dotted boxes are “high capacity options.” In other words, these are activities that can support data collection and decision-making if sufficient resources (human, financial, advanced analytical equipment, know-how, time, etc.) are available either by the program and/or by a partner.

Three case studies in [Annex I](#) provide examples of how a user might navigate the ESPT and decision trees to answer a specific question. [Annex II](#) includes a specific decision tree adapted from the President’s Malaria Initiative on selection of LLINs, including PBO LLINs and dual active ingredient (dual AI) LLINs, based on insecticide resistance data.

Baseline A. Vector occurrence and density



Example 1. Association between adult *Anopheles* nightly mean human biting rates (HBR), monthly mean precipitation, and monthly malaria incidence



Conclusions

1. *Anopheles* HBR are lowest during the driest months of the year, which are also the months with the lowest malaria incidence (January through April).
2. *Anopheles* HBR are highest in November, the wettest month of the year, and with the highest malaria incidence.
3. Increases in *Anopheles* HBR, monthly malaria incidence, and monthly mean rainfall are correlated. **The primary driver of *Anopheles* populations and malaria incidence is rainfall.**

Implications

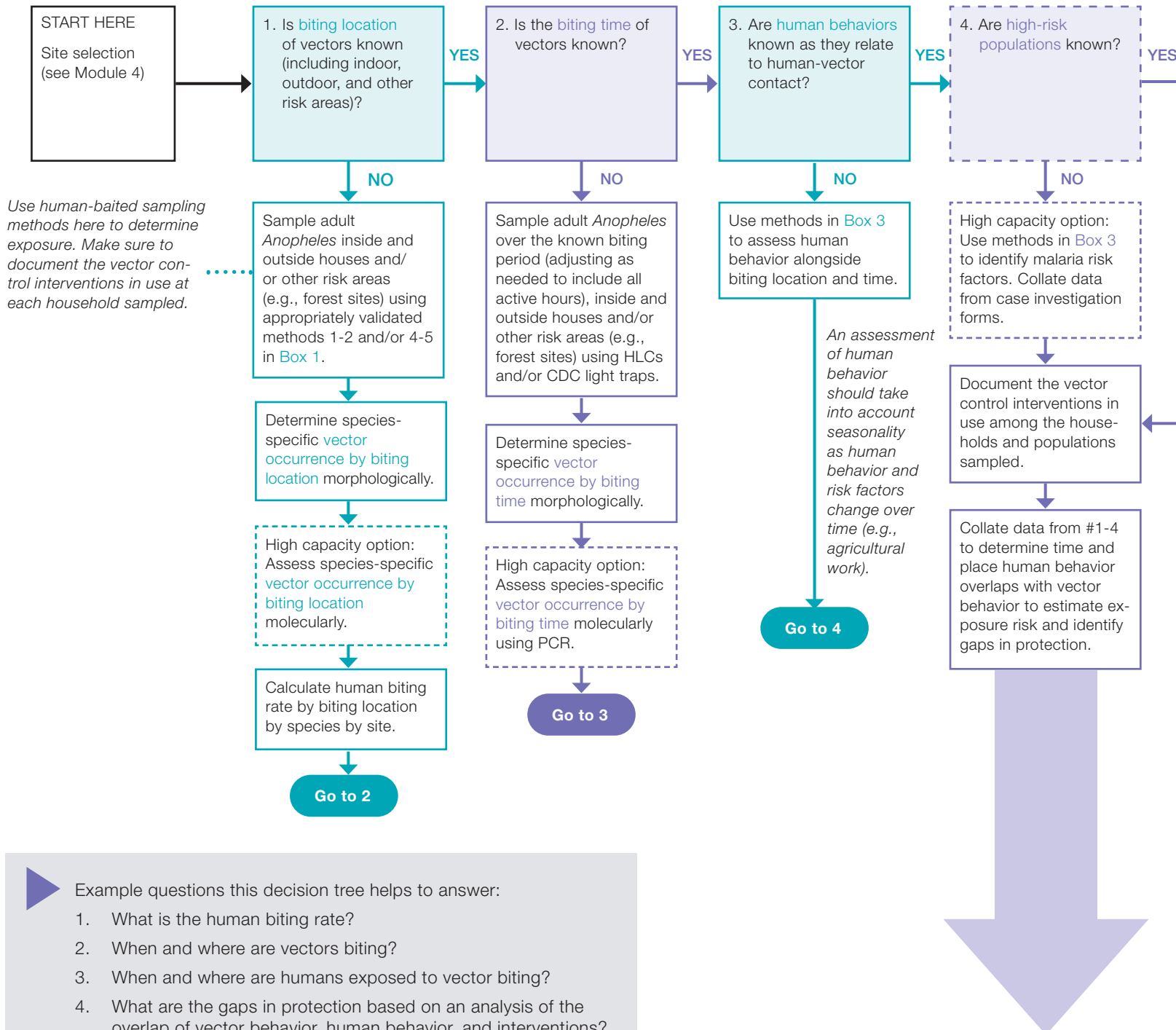
Given the correlation, timing of vector control interventions before the rains begin is critical. It is best to have multiple years of data. Based on the data presented here, vector control should be implemented in February and March to reduce HBR and the

impact on malaria transmission. The program should consider ongoing analysis of meteorological data to guide implementation.

Next steps

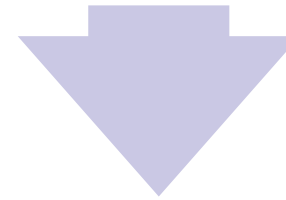
1. Species-level analysis of the *Anopheles* samples collected should be carried out to identify seasonal vector species trends (e.g., occurrence, biting behavior, resting behavior) to inform optimal intervention strategies.
2. Such data should be used to refine the time of deployment of vector control interventions to most effectively target various vector species' susceptible behaviors. E.g., if vector Species X density rises at the start of the rainy season (April), and the entomological collections showed that Species X bites primarily inside during the night, then an LLIN campaign should be carried out prior to the onset of rains, i.e., before April.

Baseline B. Vector biting behavior



Example questions this decision tree helps to answer:

1. What is the human biting rate?
2. When and where are vectors biting?
3. When and where are humans exposed to vector biting?
4. What are the gaps in protection based on an analysis of the overlap of vector behavior, human behavior, and interventions?



Early evening,
outdoor human/
vector overlap

Gap in protection.
Outdoor residual
transmission is likely
occurring outside the
protection of LLINs
and/or IRS.

See [Annex VI](#) for
supplementary inter-
ventions and WHO
recommendations.

Early evening,
indoor human/
vector overlap

**Possible gap in
protection.** Residual
transmission may be
occurring indoors
when people are
not yet asleep under
LLINs. If house is
sprayed, check
effectiveness of IRS
([Baseline C](#), [E](#), and [F](#))

See [Baseline C](#) and
[D](#) to assess effec-
tiveness of current
interventions, and
see [Annex VI](#) for
supplementary inter-
ventions and WHO
recommendations.

Late night, indoor
human/vector
overlap

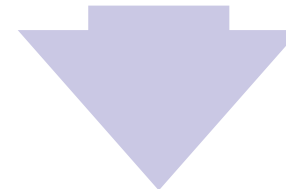
LLINs and/or IRS*
are likely appropriate
interventions *Assess
indoor resting
behavior ([Baseline C](#)).

See [Baseline C](#) and
[D](#) to confirm
effectiveness of
current interventions.

Late night, outdoor
human/vector
overlap

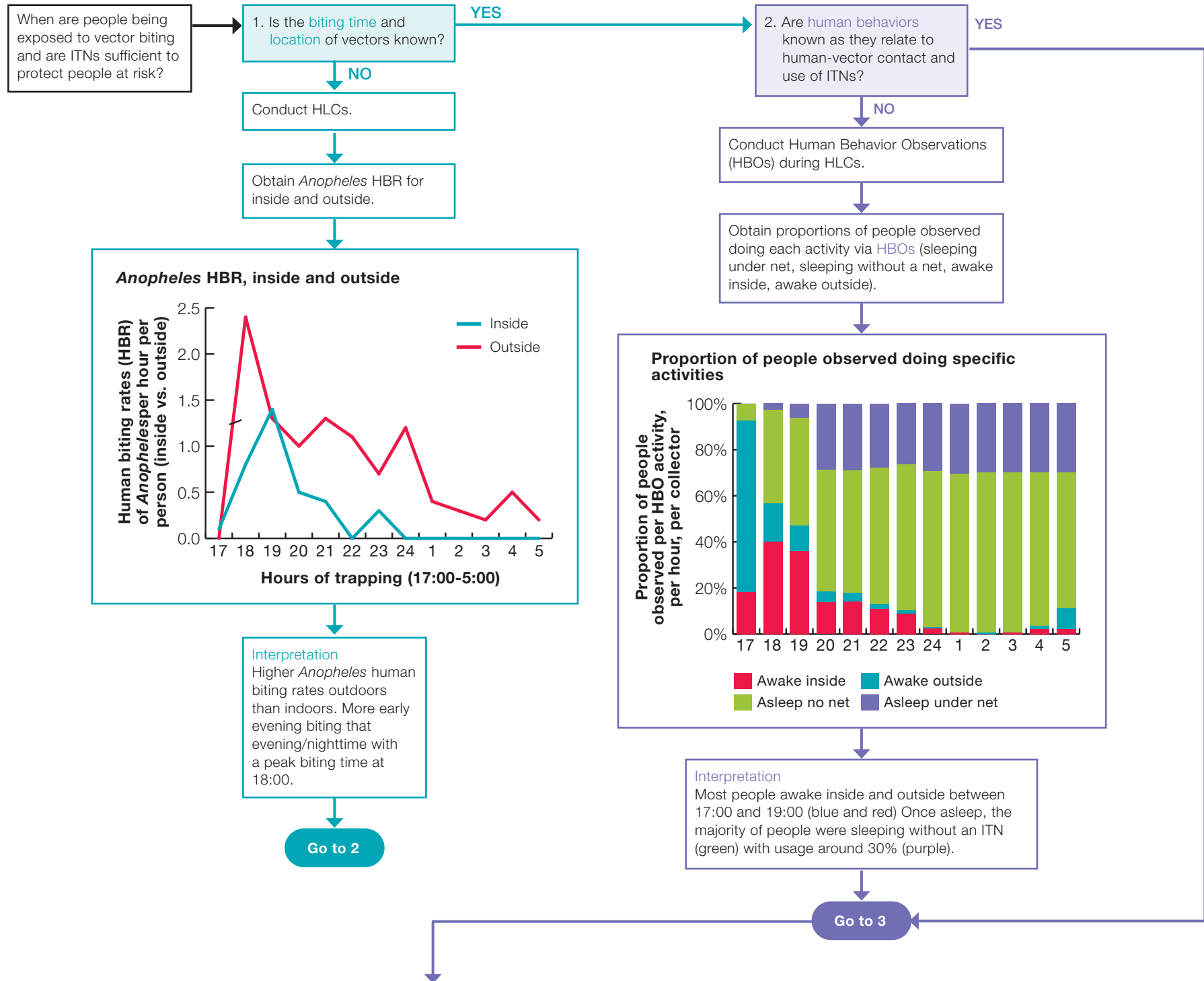
Gap in protection.
Outdoor residual
transmission is likely
occurring outside the
protection of LLINs
and/or IRS.

See [Annex VI](#) for
supplementary inter-
ventions and WHO
recommendations.



Analyze outcomes with [malaria
incidence](#) data from the same site(s)
over time to identify associations and
trends (e.g., higher malaria incidence
in areas with probable outdoor residual
transmission and gaps in protection).

Example 2: Application of Baseline B to answer the question, when and where people are exposed to vector biting?



Example 2

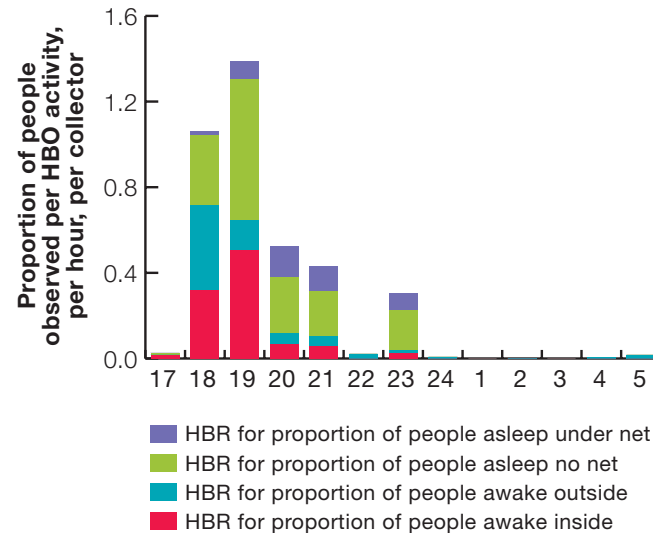
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3. What is the **adjusted HBR**, combining vector behavior with human behavior?

Use *Anopheles* HBR inside and outside (1) and HBO data (2) to obtain HBR per specific HBO activity for each collection hour (i.e., **adjusted HBR**):

$$\frac{\text{HBR inside or outside at hour X}}{\text{Proportion of people observed doing activity Y at hour X.}}$$

Adjusted biting rates for each observed activity



Interpretation

The adjusted HBR demonstrating exposure risk was highest between 18:00 and 21:00 for people awake outside and inside and asleep without a net.

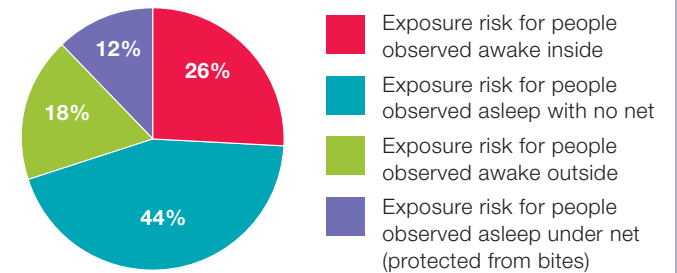
Go to 4

4. What is the **cumulative adjusted HBR**, or "exposure risk"?

Calculate the sum of hourly Adjusted HBR for each HBO activity (i.e., **cumulative adjusted HBR**):

Adjusted HBR at hour 1 + adjusted HBR at hour 2 + Convert these totals to %.

Overview of exposure risk (i.e., cumulative adjusted HBR)



Conclusions

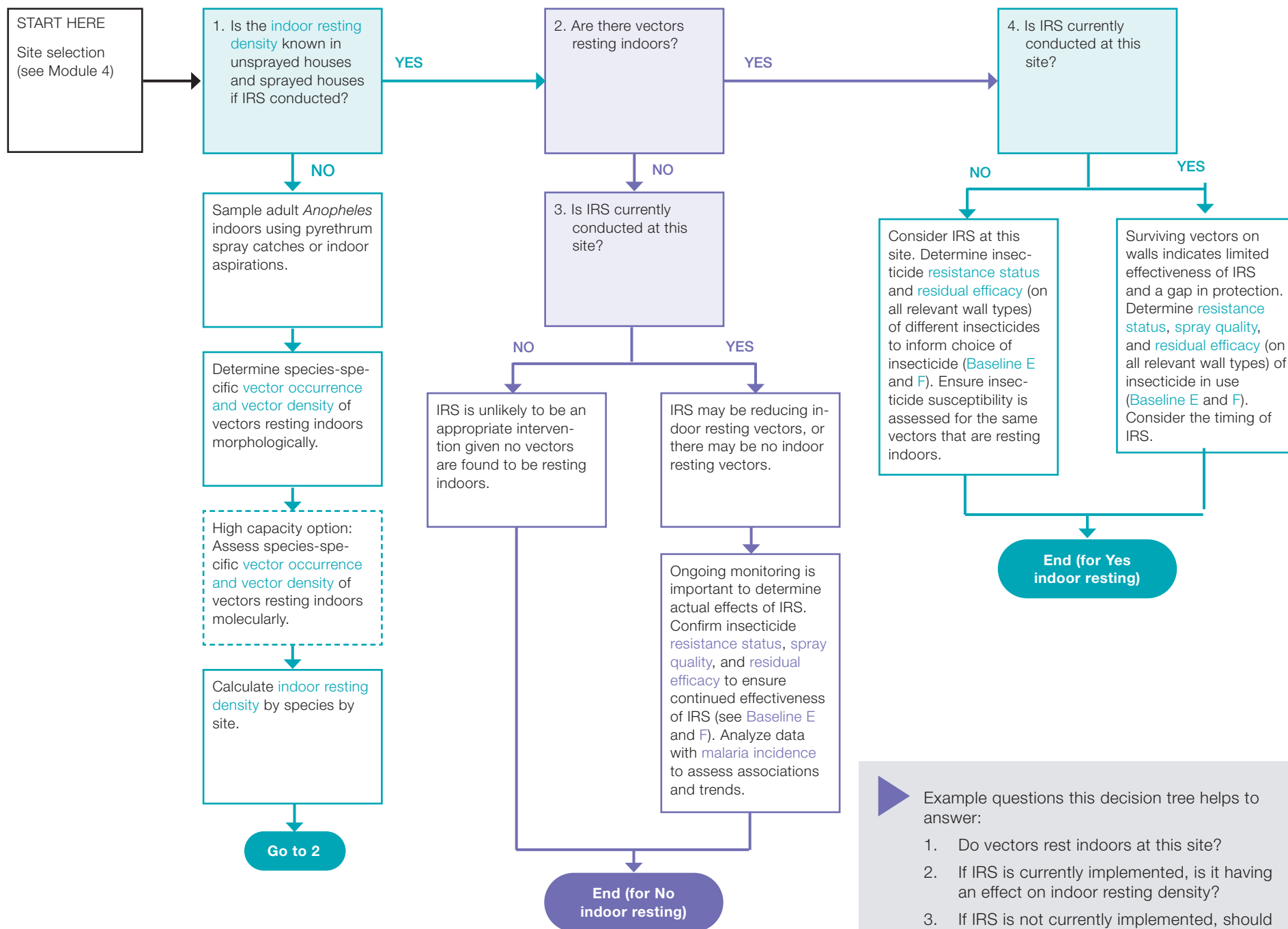
- Significant early and outdoor exposure to *Anopheles* bites.
- Human behavior influences exposure risk to bites: a) inside the house when people do not use ITNs (red and teal), and b) outside the house early at night (green). Both a and b are key gaps in protection.
- ITN usage was low.

Recommendations

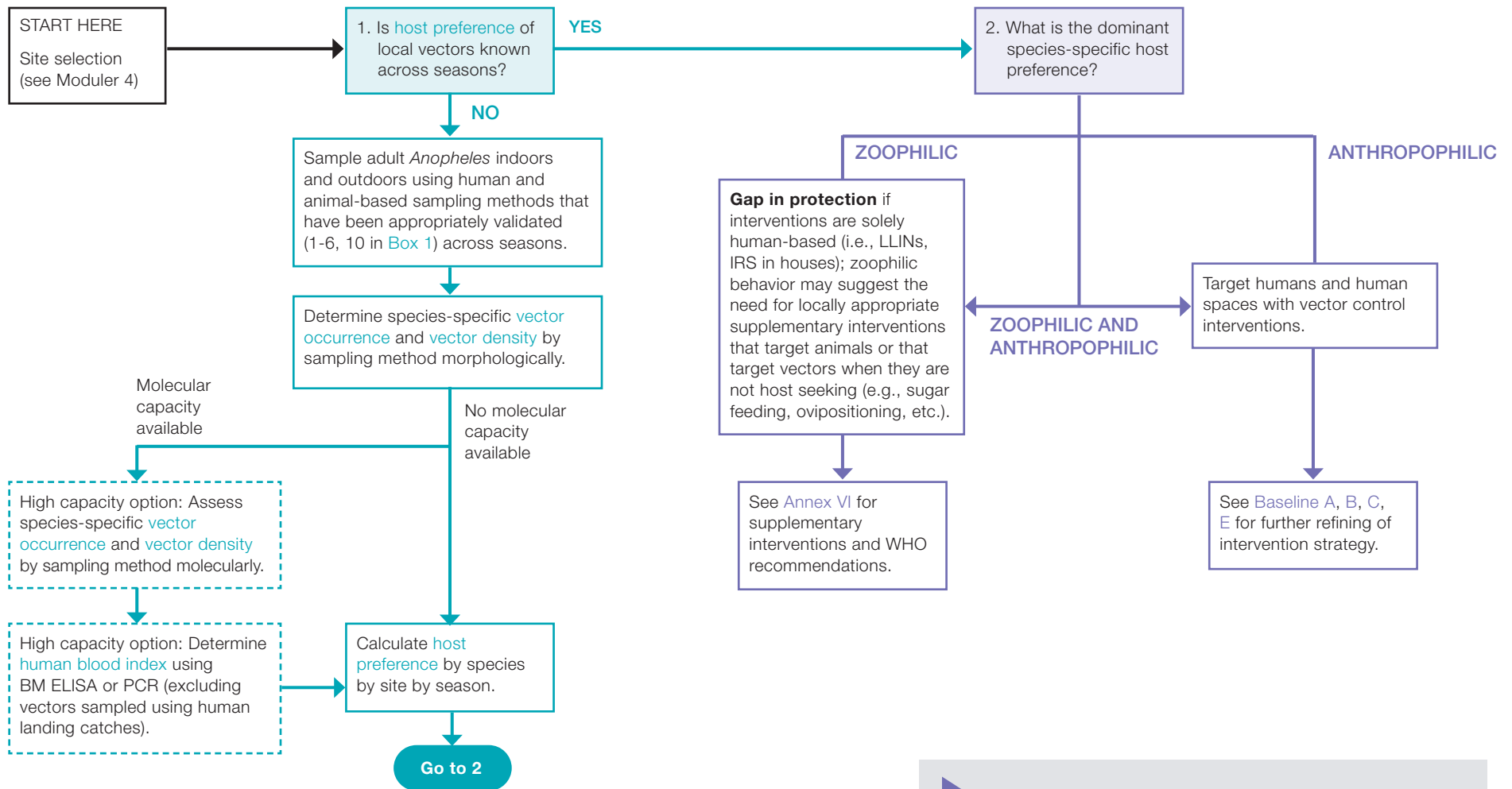
ITNs are important to reduce exposure to *Anopheles* biting indoors during sleeping hours. If low usage is associated with low coverage or access, effort should be made to improve both access to and usage of ITNs.

However, transmission will continue to occur in spaces not protected as described above. Supplemental prevention tools are required that target early outdoor biting and also indoor biting before people go to sleep under ITNs.

Baseline C. Indoor resting density



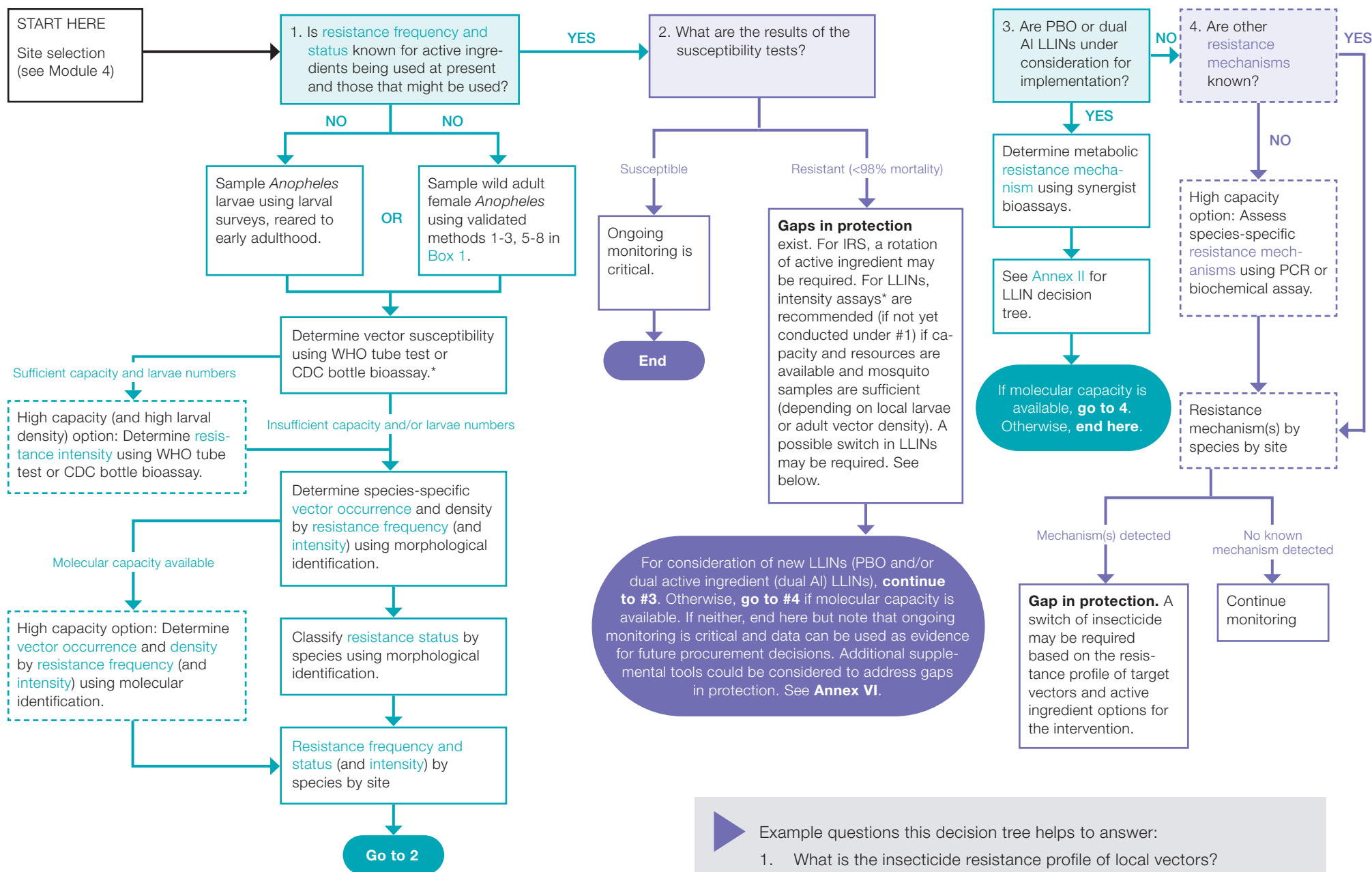
Baseline D. Host preference



Example questions this decision tree helps to answer:

1. What is the host preference for local vectors?
2. How does host preference influence the effectiveness of current vector control tools?

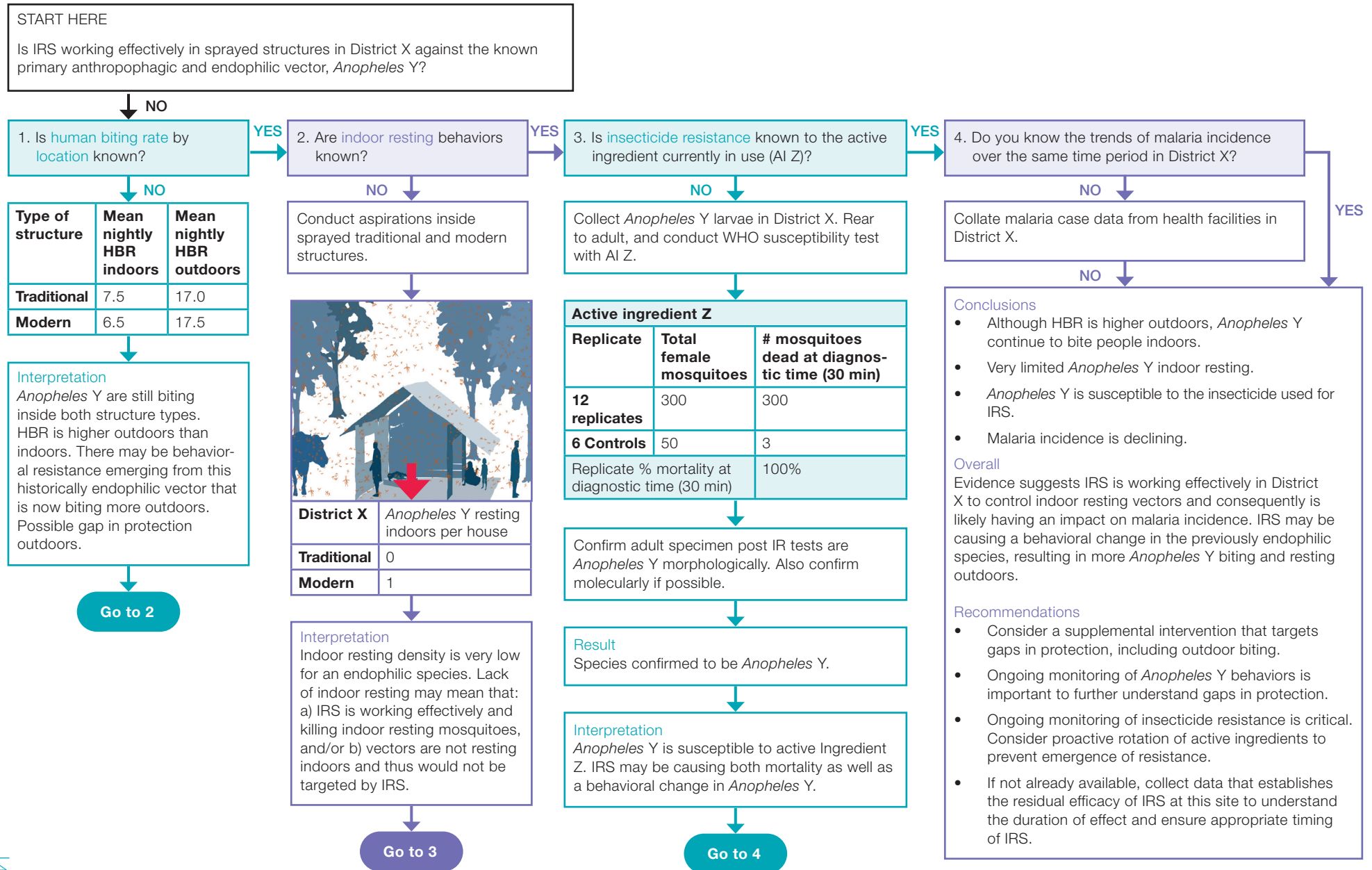
Baseline E. Insecticide resistance



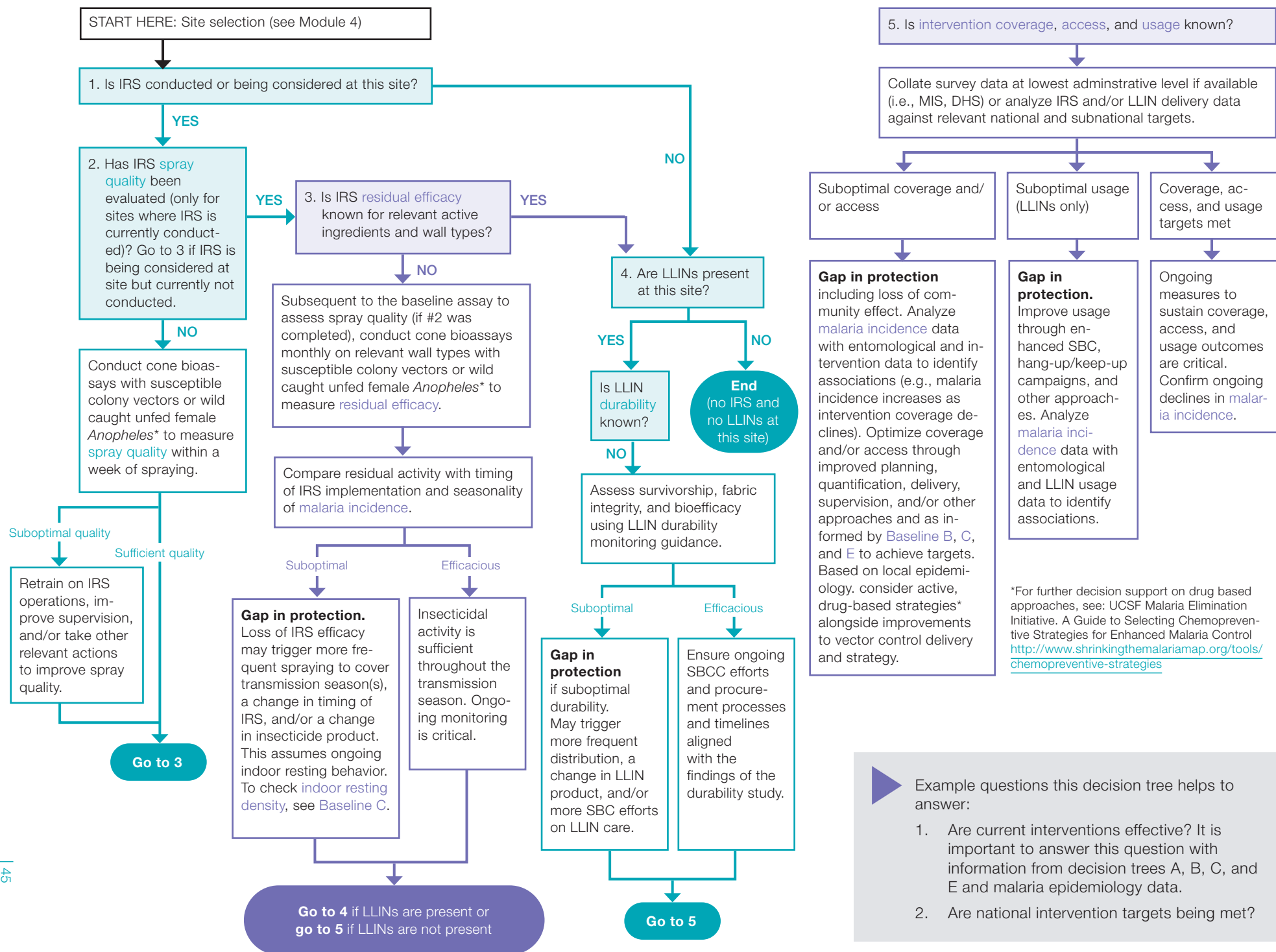
Example questions this decision tree helps to answer:

1. What is the insecticide resistance profile of local vectors?
2. What insecticide-based intervention should be considered at this site based on presence and level of insecticide resistance? Use decision tree B to help answer this question.

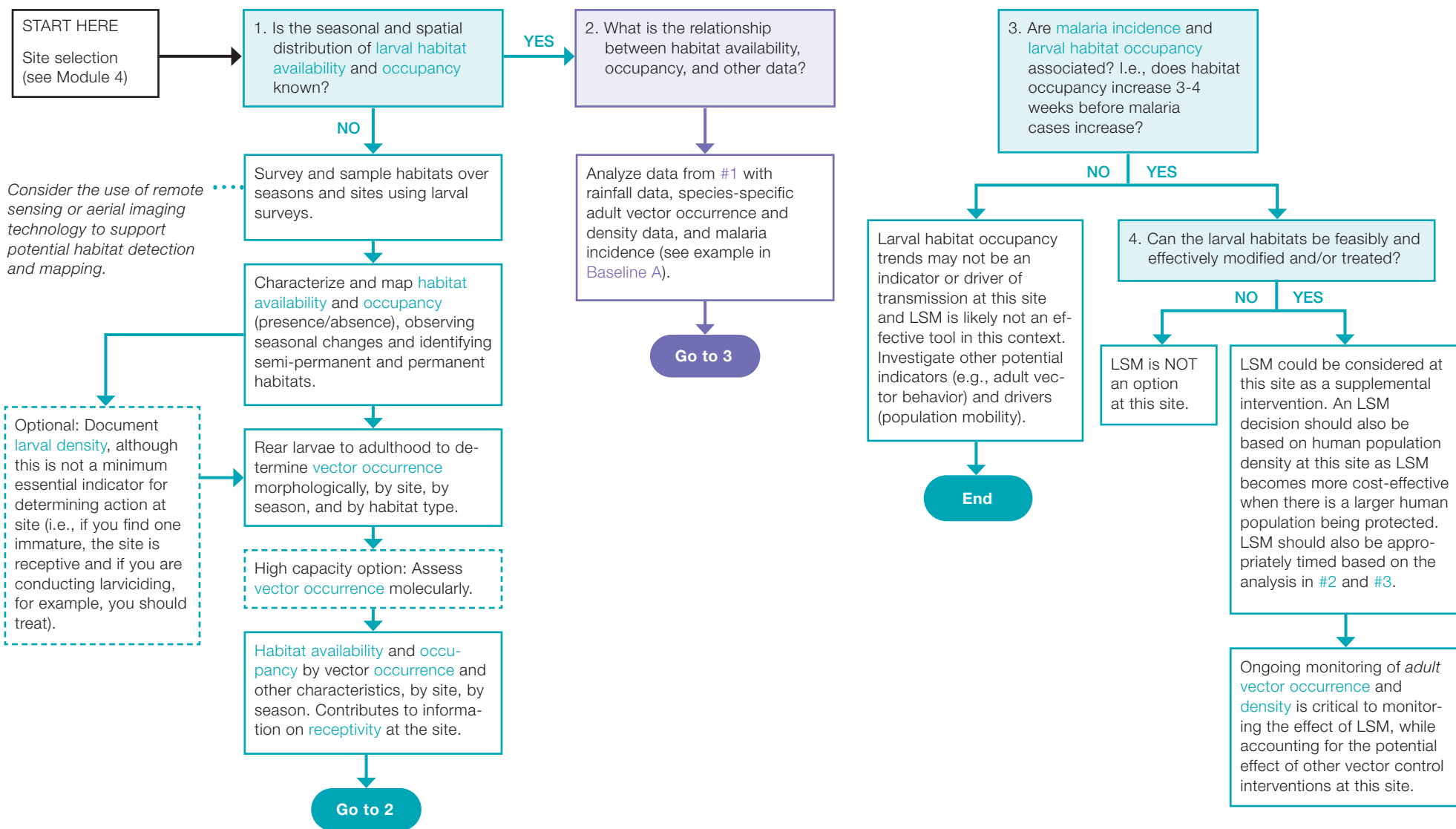
Example 3. How indoor resting density (Baseline C) and insecticide resistance (Baseline E) can be measured to answer a question about IRS effectiveness



Baseline F. Intervention efficacy



Baseline G. Larval habitat occupancy



Example questions this decision tree helps to answer:

1. Is this site receptive to malaria based on *Anopheles* larval occurrence?
2. Is LSM an option at this site?
3. If LSM is conducted at this site, is it effective?

Module 8. Decision Trees for Routine Surveys and Monitoring Receptivity

Below are five decision trees for routine monitoring of priority indicators:

- Routine A. Vector occurrence and density
- Routine B. Vector biting behavior
- Routine C. Indoor resting density
- Routine D. Insecticide resistance
- Routine E. Larval habitat occupancy

These decision trees can be used for:

1. Routine surveys at sentinel sites to monitor changes in vector populations over time, monitor the impact of interventions on local vectors, and identify emerging gaps in protection
2. Where capacity exists, routine surveys in active foci in very low transmission settings with similar objectives as above

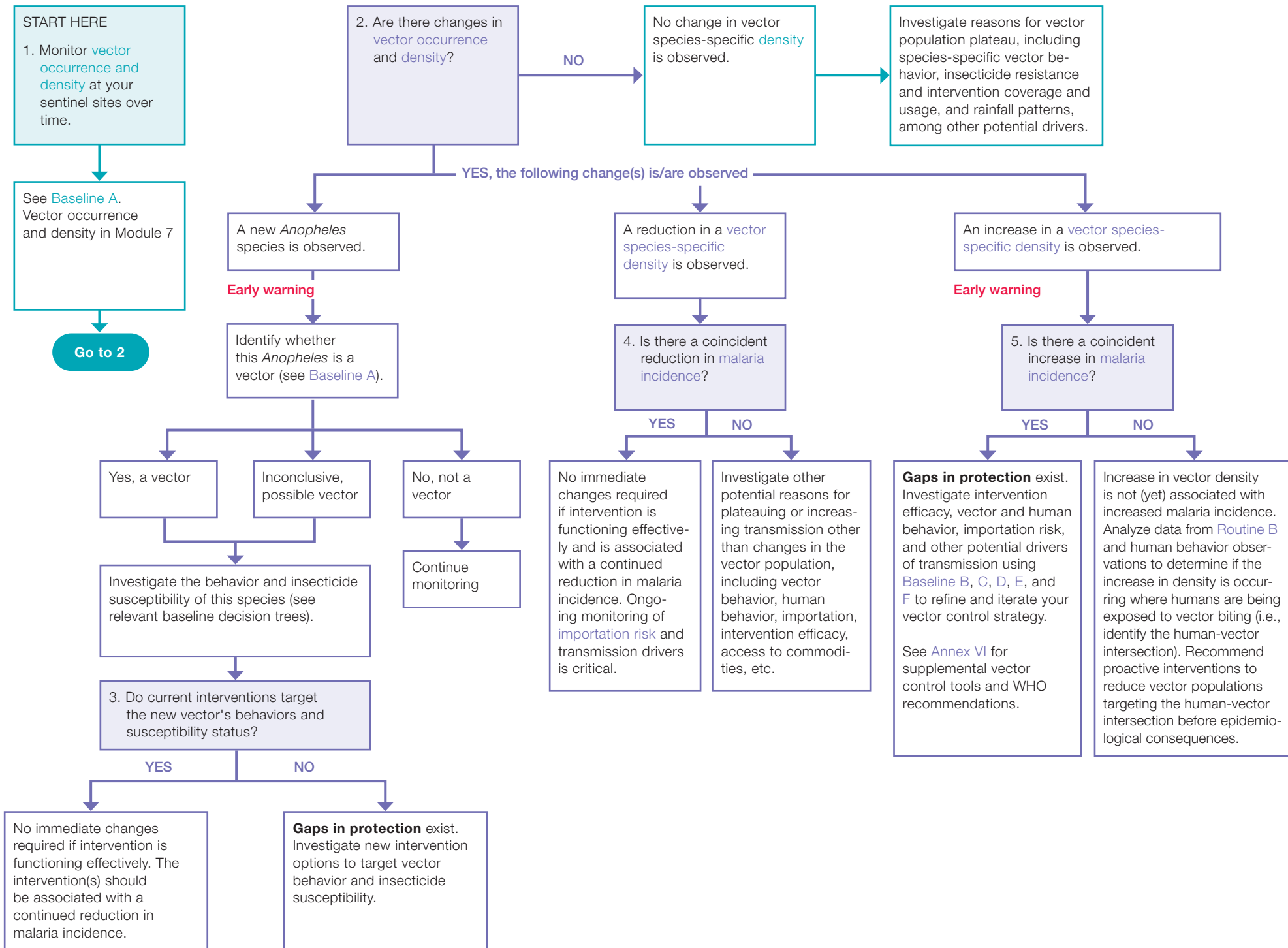
The decision trees refer the user to Baseline trees for collecting data for specific indicators. The user should then return to these *routine* trees to consider the implications of results from the routine monitoring activities and to read recommendations for action based on the results.

The sixth decision tree below is for routine monitoring at one or multiple sentinel sites in areas **preventing reestablishment** of transmission. These are areas with no current local malaria transmission but with a recent history of local transmission and risk of importation of parasites.

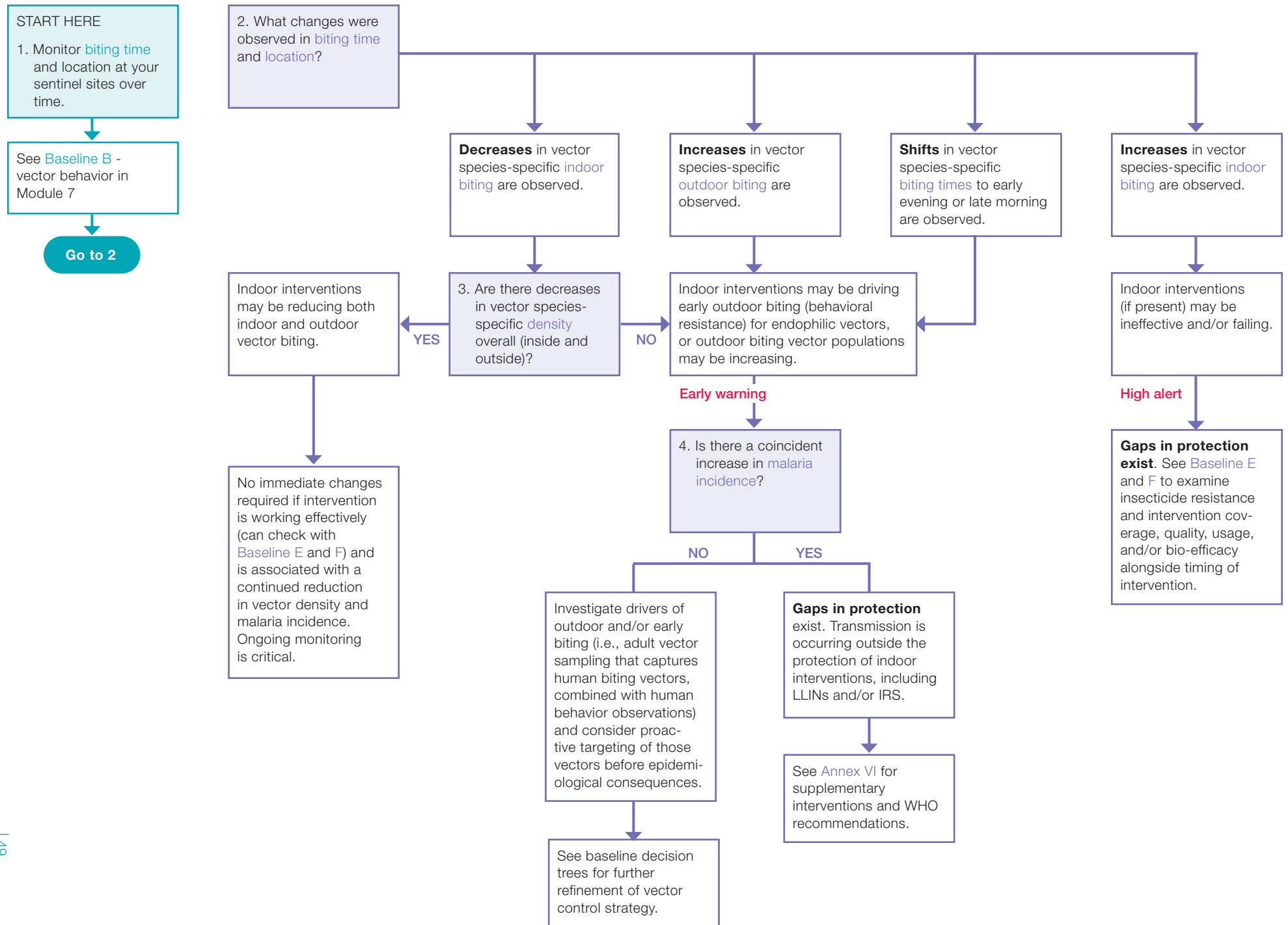
Entomological surveillance in prevention of reestablishment (POR) settings is very context and capacity dependent. In moderate transmission countries with areas that have eliminated local transmission, the focus for entomological surveillance should likely remain in areas with ongoing transmission given available resources. However, in countries with low or very low transmission, it may be important to establish sentinel sites in areas that have recently eliminated to monitor receptivity especially if there is ongoing parasite importation risk. In countries that have eliminated malaria, establishing sentinel sites in previously endemic areas to monitor key indicators can guide prevention of reestablishment strategies and outbreak response plans.

In all six decision trees, there are *early warnings* and *high alerts*, indicating a particular result is alarming and should be followed up with further investigation and action. *High alert* should lead to immediate intervention as suggested in the trees.

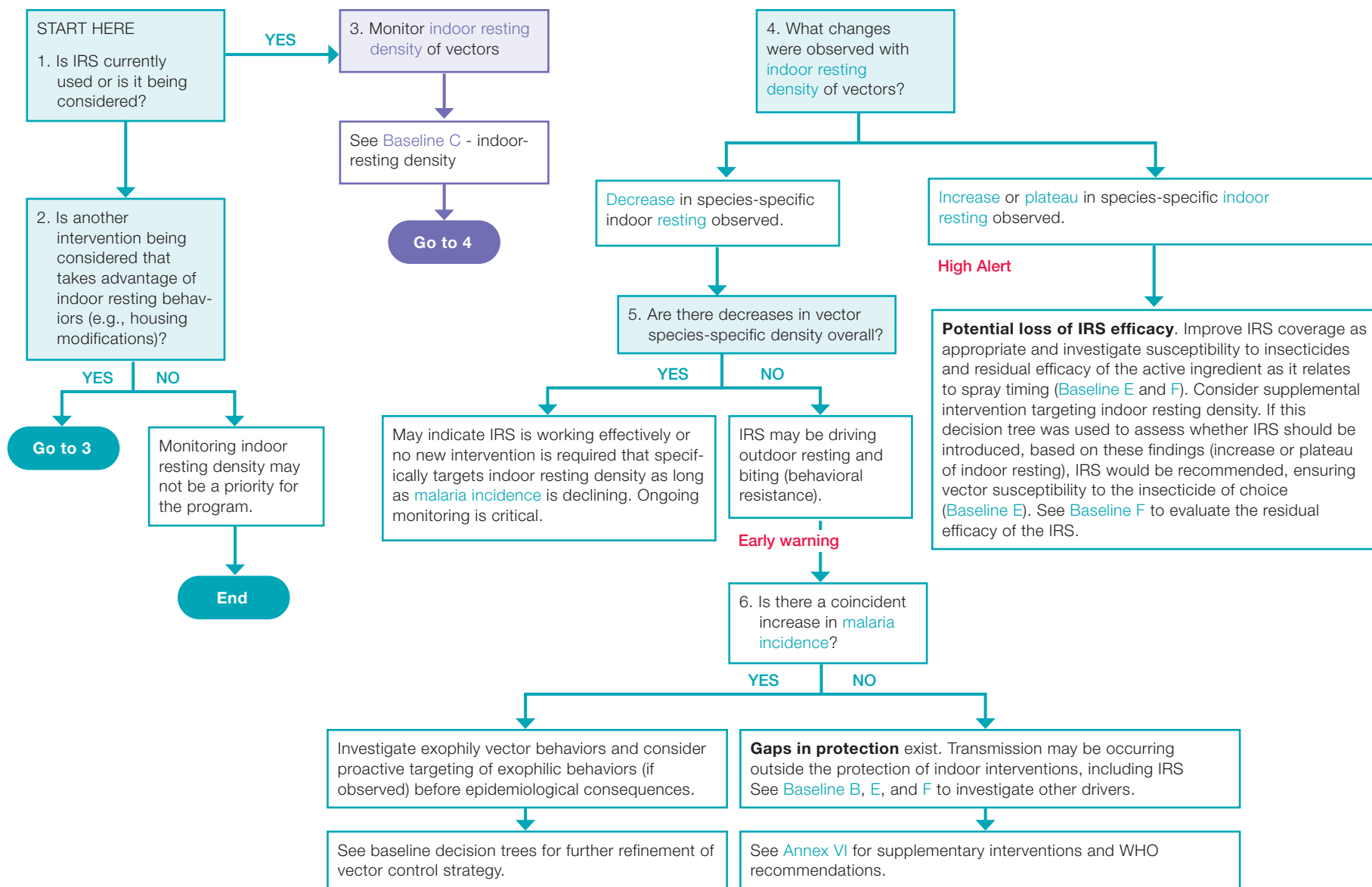
Routine A. Vector occurrence and density



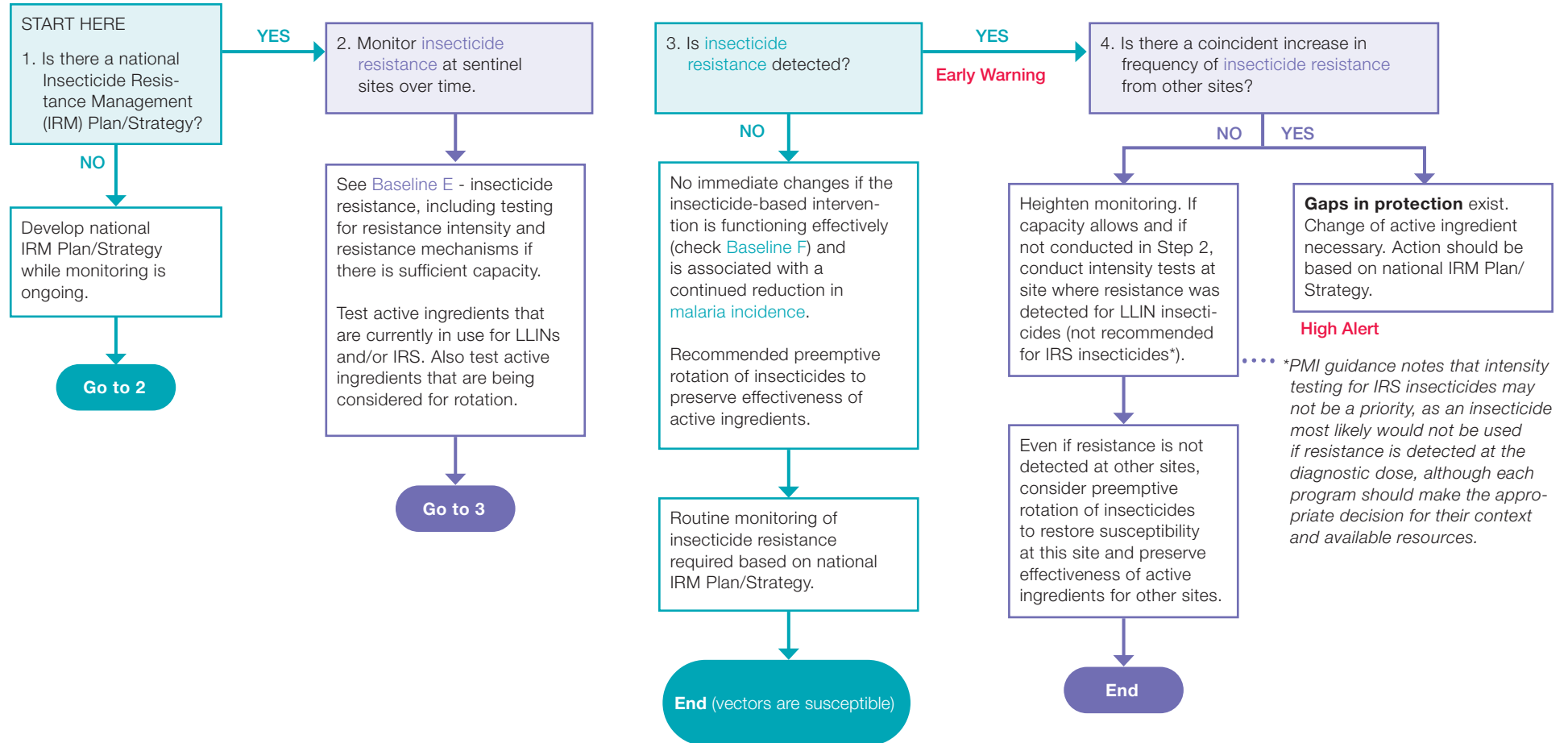
Routine B. Vector biting behavior



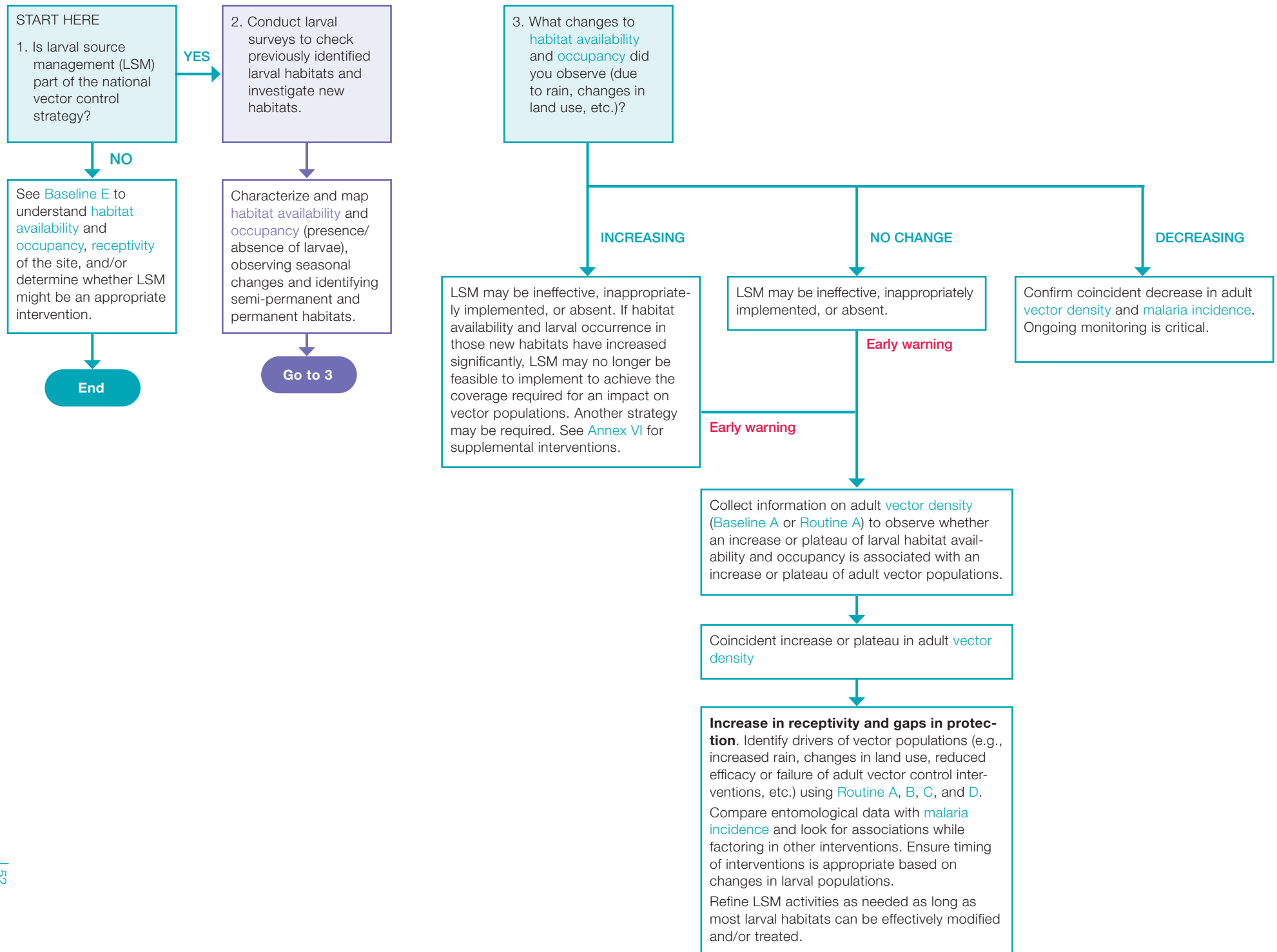
Routine C. Indoor resting density



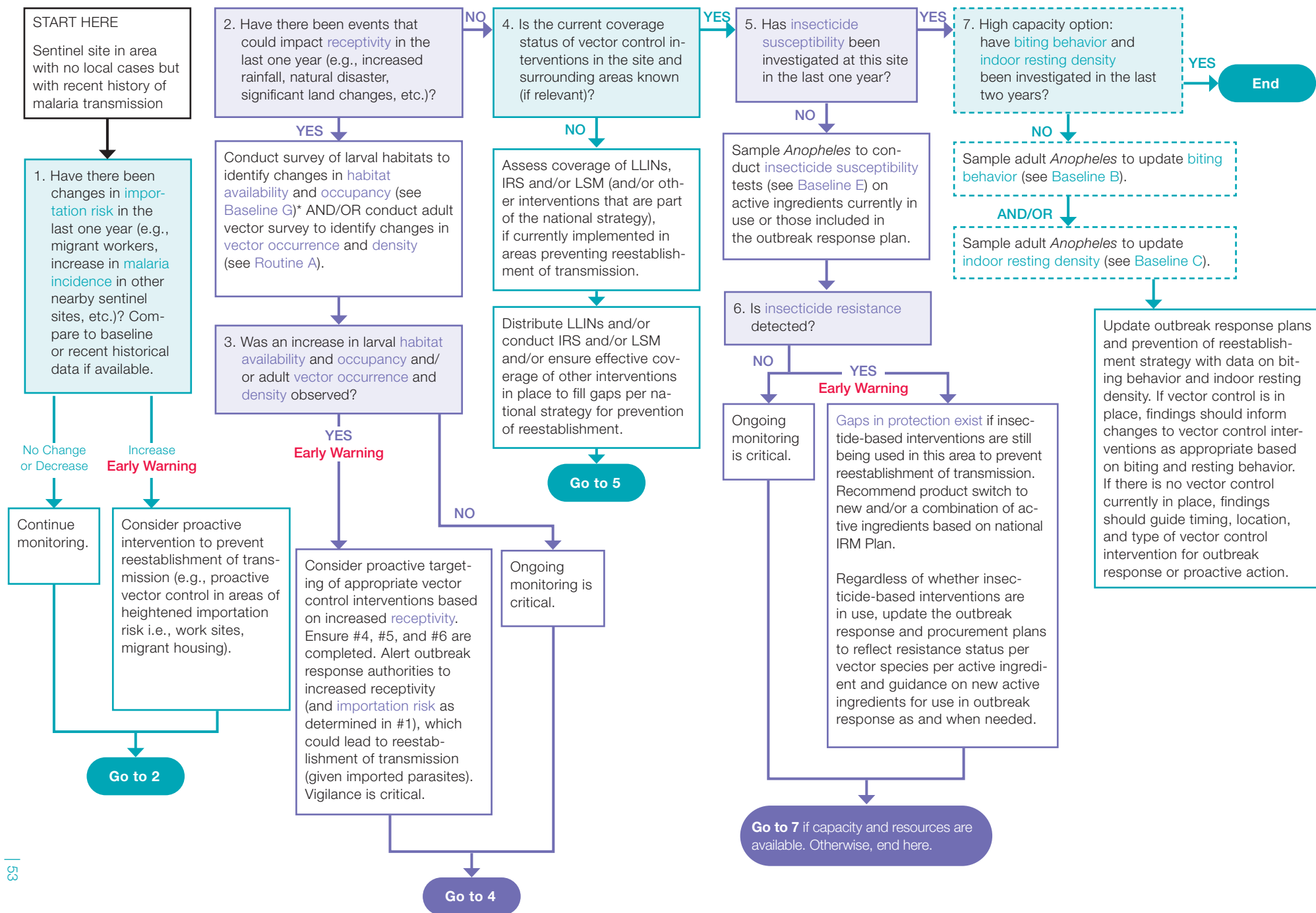
Routine D. Insecticide resistance



Routine E. Larval habitat occupancy



Prevention of Reestablishment (POR)

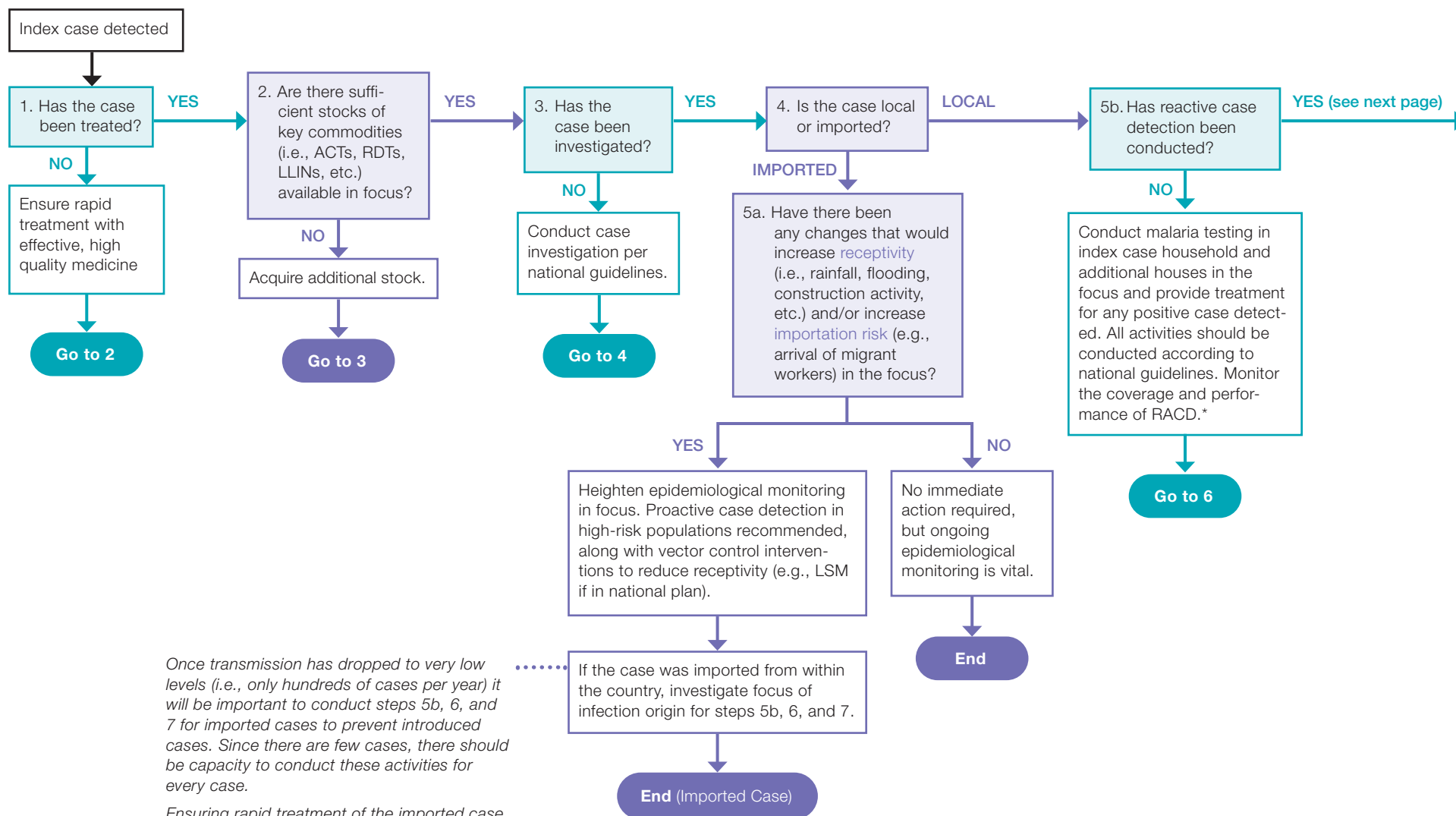


Module 9. Decision Trees for Focus Investigation

The ESPT includes two decision trees for focus investigation: Phase 1 and Phase 2. Phase 1 should be used during all foci investigations to gather priority epidemiological, entomological, environmental, and intervention data to inform a tailored and rapid response to stop onward transmission. Phase 2 should be used only annually to investigate entomological drivers of transmission in active foci using indicators described above for baseline surveys. This separation of activities in Phase 1 and

Phase 2 was done with recognition of the limited entomological capacity available for foci investigation in most countries. More importantly, separating the activities helps to clarify the relevance of data to decision-making in a rapid foci response, Phase 1, versus Phase 2 that gives a broader understanding of what might be causing continued transmission in an active focus where a dedicated entomological surveillance team might visit once a year to investigate.

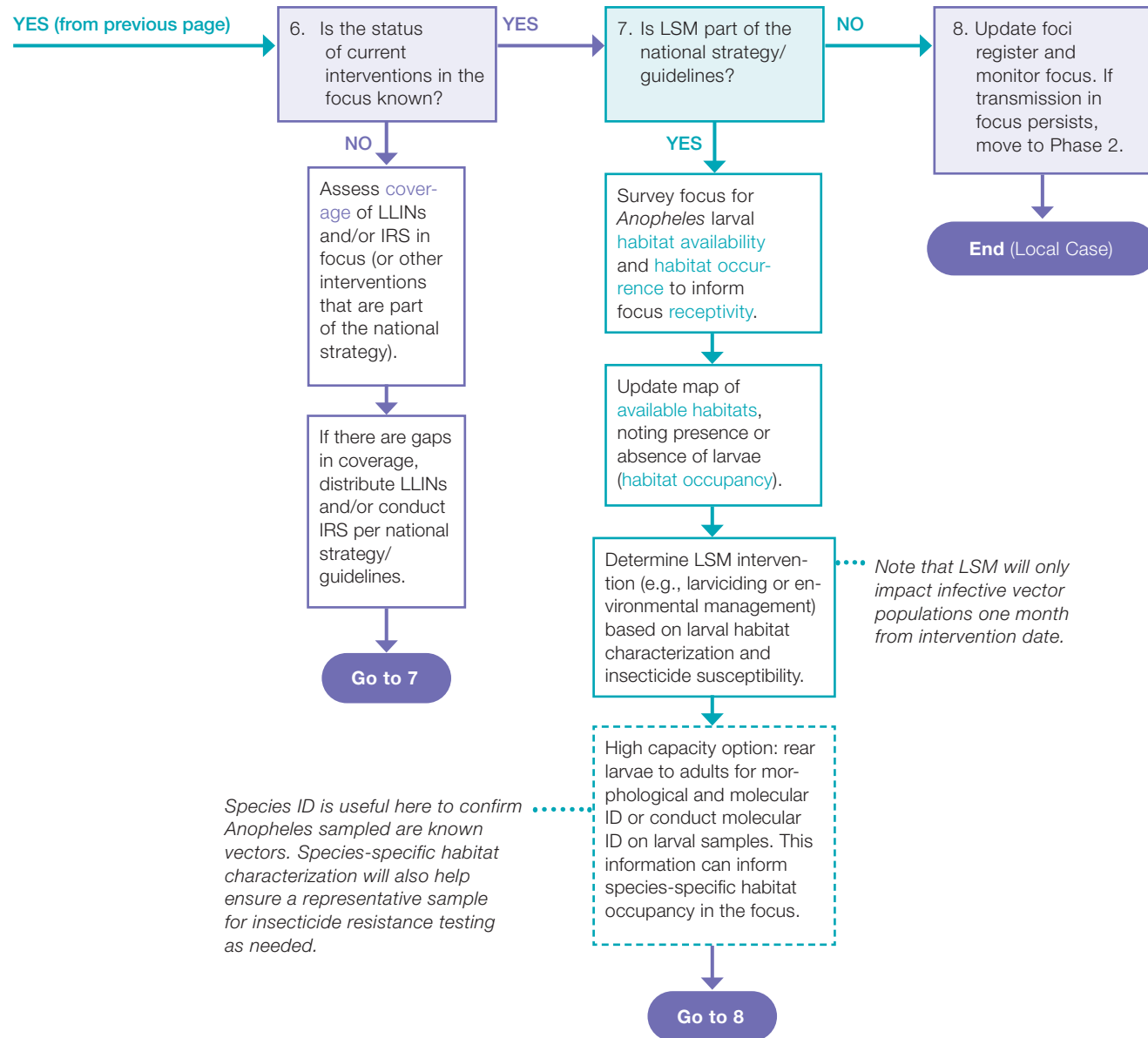
Focus investigation: Phase I



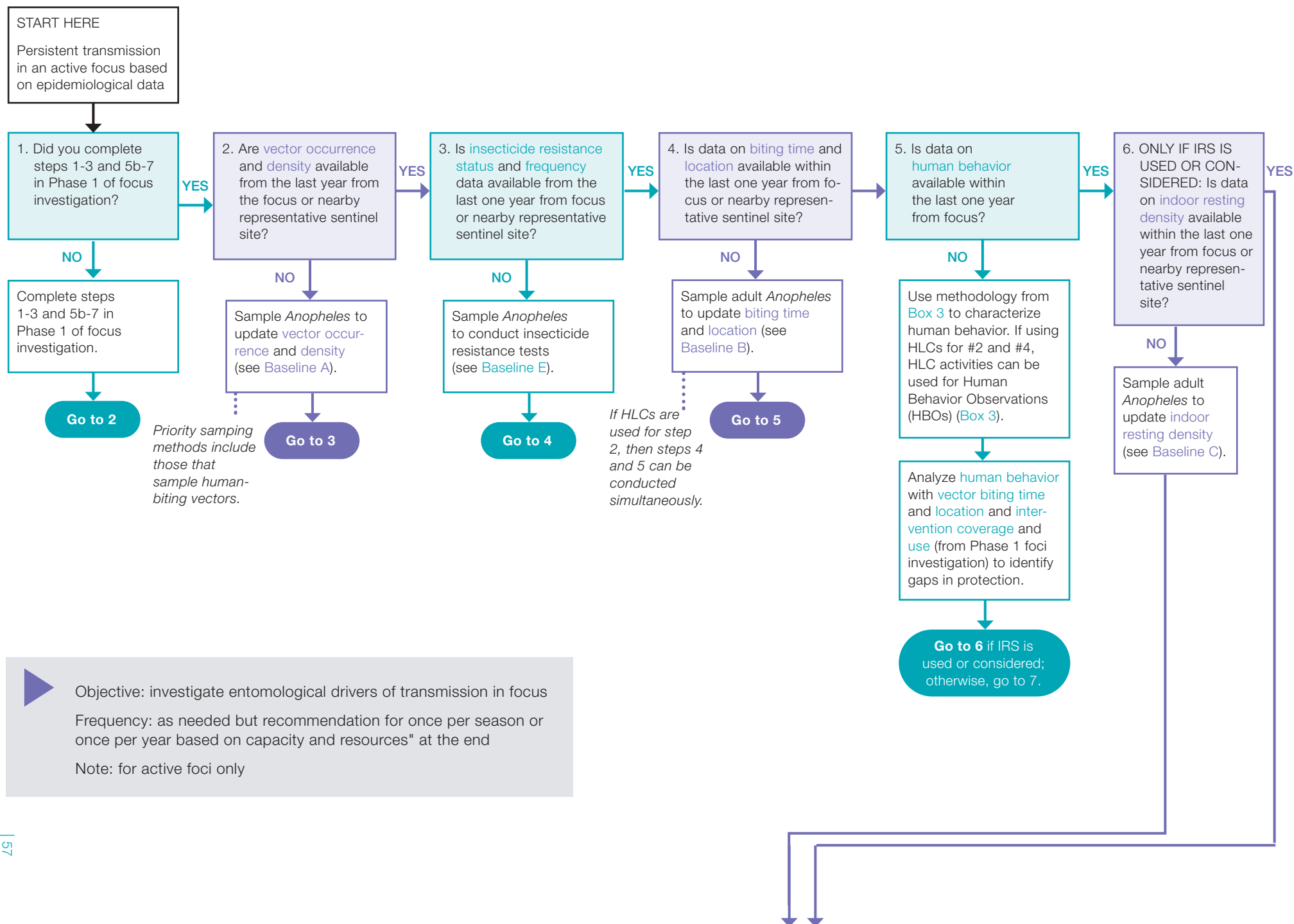
Once transmission has dropped to very low levels (i.e., only hundreds of cases per year) it will be important to conduct steps 5b, 6, and 7 for imported cases to prevent introduced cases. Since there are few cases, there should be capacity to conduct these activities for every case.

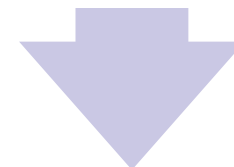
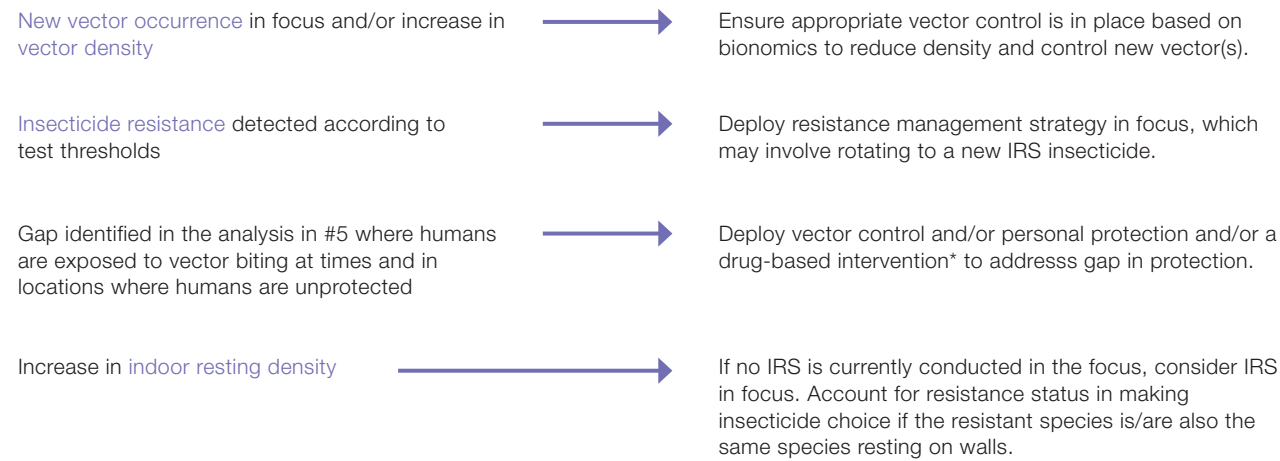
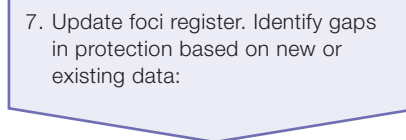
Ensuring rapid treatment of the imported case is the most critical at this point.

*For further support on M&E of RACD, see: Malaria Elimination Initiative. Reactive Case Detection (RACD) Monitoring & Evaluation Tool. San Francisco: The Global Health Group, University of California, San Francisco. <http://www.shrinkingthemalariamap.org/tools/reactive-case-detection-monitoring-evaluation-tool>



Focus investigation: Phase 2





Adjust intervention deployment as needed based on entomological, ecological (seasonality), and epidemiological indicators.

Consider drug-based intervention in addition to vector control to attack the parasite reservoir in the focus.*

Maintain surveillance and focal strategy until transmission is interrupted. Conduct ongoing monitoring of gaps in protection to ensure gaps are effectively addressed.

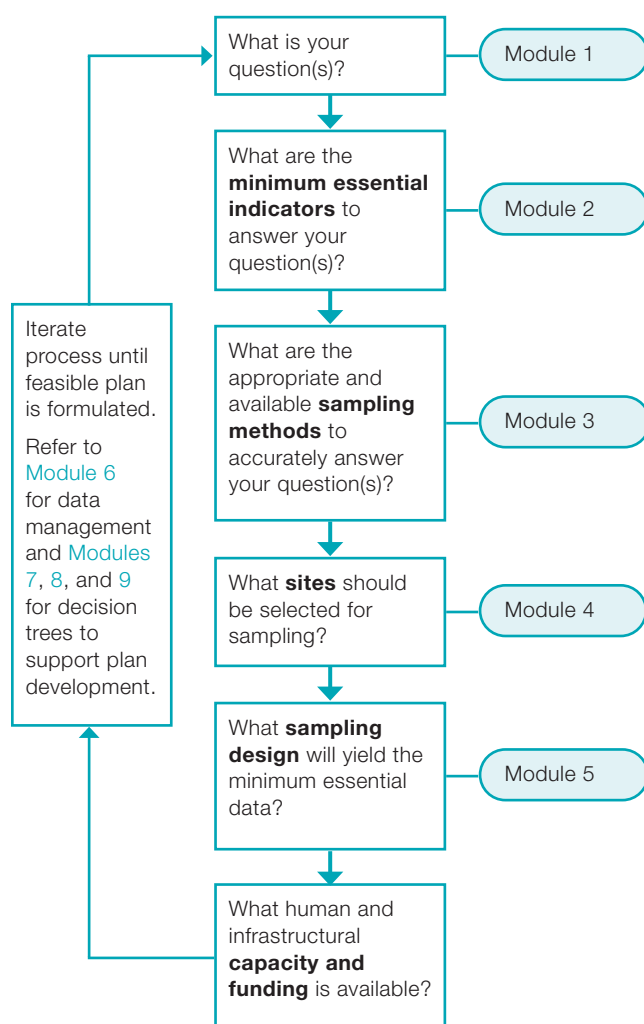
*For further decision support on drug-based approaches, please see: UCSF Malaria Elimination Initiative. A Guide to Selecting Chemopreventive Strategies for Enhanced Malaria Control <http://www.shrinkingthemalariamap.org/tools/chemopreventive-strategies>

Annex I

Step-by-Step Examples: How to Use the ESPT to Answer Specific Questions

Steps 1 through 5 of the three example guides follow the workflow of the Navigation Tree from the [Background](#) chapter, included again below.

Figure 1. Navigating the ESPT



Example A

Step 1: Define your question(s), **Module 1.**

Primary question: Should we use IRS in Area X?

Step 2: Select the relevant indicators to address your question(s), **Module 2.**

Select indicators that will answer this question. For [Example A](#), indicators would include:

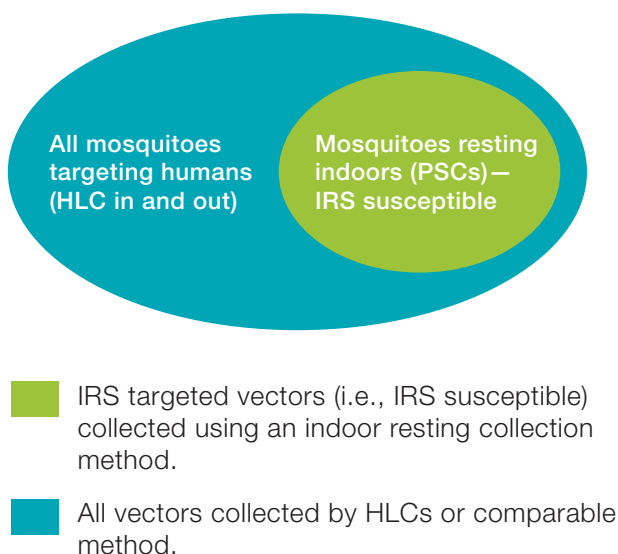
1. **Vector occurrence** and **vector density** to examine the presence of specific vector species and the relative vector composition,
2. **Indoor resting density** to examine the susceptibility of vectors to IRS based on their resting behaviors, and
3. **Insecticide resistance status** to examine the susceptibility of vectors to insecticide being considered for IRS.

Step 3: Determine appropriate sampling methods, **Module 3.**

Link each of the **indicators** listed in Step 2 to specific **entomological sampling methods** that will generate data about the proportion of vectors that might be affected by IRS. Note that some methods will generate data for multiple indicators:

1. **Vector occurrence** and **vector density**:
 - a. **Use an indoor resting collection** method, either pyrethrum spray catches (PSCs) or indoor aspirations, to provide information on vectors that rest on walls. In this example, PSCs were selected for use (Figure 2).
 - b. And use **HLCs** (or a proxy that can answer the same question) both indoors and outdoors to characterize overall mosquitoes biting indoors and outdoors. In this example, HLCs were selected for use. Figure 2 below represents all vectors present at the site that are collected by both HLCs and PSCs, versus the vectors collected by PSCs only.

Figure 2. Representation of all vectors at a site (collected by HLCs and PSCs) in blue, relative to those (collected by PSCs) that are susceptible to IRS, in green.



2. **Indoor resting density:** use an **indoor resting collection method** such as PSCs or indoor aspirations. In this example, PSCs were selected for use.

Note: Some vectors may rest on walls and leave the house before morning PSCs and would be missed during sampling. All night IAs or WETs may include these vectors.

3. **Insecticide resistance status:** use a **vector collection method** such as larval surveys or adult collections and a **resistance testing method** such as WHO tube tests or CDC bottle bioassays. In this example, larval surveys and WHO tube tests were selected.

Note: Larval samples reared to adulthood for insecticide resistance testing is the standard technique, but it is important to ensure that larval catches represent the indoor resting population—the primary target of IRS. Species compositions in larval sampling should be compared to adult vectors captured resting indoors to ensure that data on resistance reflects the IRS-targeted vector population.

Step 4: Select sites, [Module 4](#).

Sampling sites should be within, and representative of house types in Area X, and selected based on the entomological indicators listed in Step 2, as well as available human and financial resources. In this example, four villages where IRS is being considered

were selected and each village was considered a separate site (i.e., one village = one site).

Step 5: Formulate sampling design, [Module 5](#).

After Steps 1–4 are completed, the sampling design below should be formulated for each site/village:

- **HLC indoors and outdoors:** inside and outside 4 sentinel houses over 5 nights per month throughout the 5-month transmission season.
- **PSCs:** inside 10 randomly selected houses (excluding HLC houses), once per month throughout the 5-month transmission season. Different houses were selected each time to prevent residual PSC insecticide from affecting catches.
- **Larval sampling for resistance testing:** larval sampling conducted in all identified larval sites throughout the transmission season (alongside the HLCs and PSCs) after confirming (by morphological or molecular identification) that sampling would reflect indoor resting vector populations (i.e., the same vector species).

Step 6: Reference the decision trees, [Module 7](#).

Baseline trees A: vector occurrence and density, C: indoor resting density, and E: insecticide resistance can support the process of determining appropriate sampling methods, workflow, and design.

Step 7: Carry out the fieldwork.

Step 8: Process, collate, and analyze entomological data, [Module 6](#).

Step 9: Interpret results.

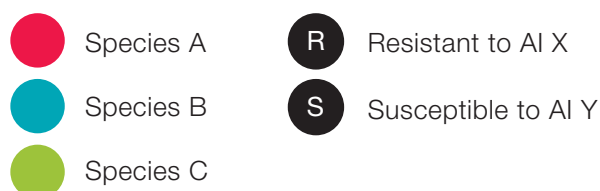
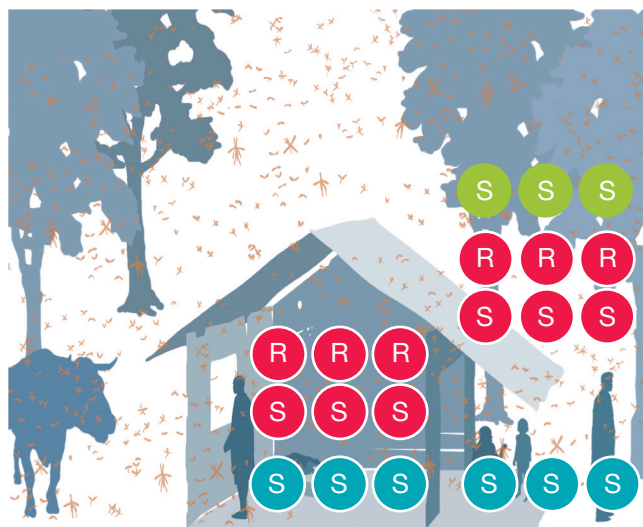
*Note: If molecular capacity is available, validation of species using molecular techniques, in addition to morphological identification for all collections, especially after resistance testing, is important to understand species-specific resistance and possible species-specific effects of IRS, if IRS was implemented in Area X. Inferring results based on species complexes (e.g., *An. gambiae* s.l.) risks inaccurate conclusions about potential effectiveness of IRS.*

1. **Vector occurrence and density:** HLC and PSC collections morphologically identified 2 species. PCR identified these to 3 species: Species A (a primary vector found in large numbers indoors), Species B (found indoors and outdoors in smaller numbers), and Species C (found outdoors only in large numbers) ([Table 1](#)).

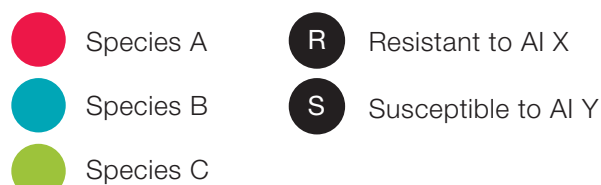
Table 1. Summary of results

Vector species	Relative density, collected indoors/outdoors	Resting status	Resistance status
Species A	High, indoors	Indoor resting	Resistant to insecticide X Susceptible to insecticide Y
Species B	Low, indoors and outdoors	Indoor resting	Susceptible to insecticide Y
Species C	High, outdoors	Not found resting indoors	Susceptible to insecticide Y

2. **Indoor resting density:** PSC data demonstrated the presence on 1 morphologically identified species resting on walls. PCR distinguished the 1 species as 2 species: Species A and Species B. (Table 1)
3. **Insecticide resistance status:** molecular identification after WHO tube tests (Table 1) confirmed:
 - a. Species A resistant to insecticide X but susceptible to insecticide Y;
 - b. Species B and C susceptible to both insecticides X and Y.

Figure 2. Indoor and outdoor HLCs + IR status

*The number of bubbles represents relative density of the different species

Figure 3. PSCs and IR status

*The number of bubbles represents relative density of the different species

Step 10: Using results described in Step 9 to answer the question, should we use IRS in Area X?

1. Likely efficacy of IRS:

- a. Species A and B rest indoors and are susceptible to insecticide Y, thus Species A and B would be affected by IRS with insecticide Y.
- b. Species C likely not affected by IRS since Species C, found in HLCs was not found resting indoors in PSCs.

2. **Remaining gaps in protection:** Gaps would include exposure of humans outdoors since all species were found biting outdoors. Transmission by Species C may be unaffected by IRS.
3. **Way forward:** Monitoring of indoor resting, indoor and outdoor biting, and insecticide resistance is important to evaluate the effects of IRS, including changes in vector behaviors and insecticide resistance.

Example B

Step 1: Define your questions, [Module 1](#).

Primary question: What are the vectors in Area Y?

Step 2: Select the relevant indicators to address your question, [Module 2](#).

Select indicators that will answer this question. For [Example B](#), indicators would include:

1. **Vector occurrence** to examine the presence of specific vector species,
2. **Vector density** to examine the relative vector composition and potential contribution to disease,
3. **Seasonality** to document temporal changes in vector populations.

Step 3: Determine appropriate sampling methods, [Module 3](#).

Link each of the indicators listed in Step 2 to specific entomological sampling methods that will generate data about vectors in Area Y:

1. **Vector occurrence** and **vector density** using the following methods:
 - a. **HLCs** to sample vectors that bite humans. Sampling may be performed in 3 representative risk areas within Area Y as appropriate: a) indoors, b) outdoors in the peri-domestic area, and c) outdoors in non-domestic risk areas (e.g., forest work sites) OR HLC proxy, which includes methods including the CDC light trap. Before using a proxy method, it is important to understand how well the HLC proxy correlates to HLC collections. In this example, HLCs were selected for use, as well as
 - b. **Animal-baited trap** to sample animal-biting vectors that still contribute to malaria transmission despite their zoophagic preferences to more comprehensively answer the question, what are the vectors in Area Y? In this example, we're interested in all vectors present.

2. **Seasonality:** to characterize vector populations over the course of one year, collections should be performed **at several time points** throughout the year based on available capacity and resources.

Step 4: Select sites, [Module 4](#).

Four villages were selected based on a stratification of Area Y that considered local epidemiology, ecology, and intervention coverage. Four strata were identified and thus one village per stratum was selected for sampling.

Step 5: Formulate sampling design, [Module 5](#).

After Steps 1 – 4 were completed, the sampling design below was formulated:

1. **HLC indoors and outdoors:** inside and outside 5 sentinel houses in each of the 4 villages as well as 3 forest-based work sites in Area Y; sampling conducted over 5 nights every two months
2. Sampling methods to trap zoophagic or animal-biting vectors were not used in this example due to budgetary constraints. Therefore, only the proportion of vectors that feed on humans were sampled.

Step 6: Reference decision trees, [Module 7](#).

Baseline tree A: Vector occurrence and density can support the process of determining appropriate sampling methods, workflow, and design.

Step 7: Carry out the fieldwork.

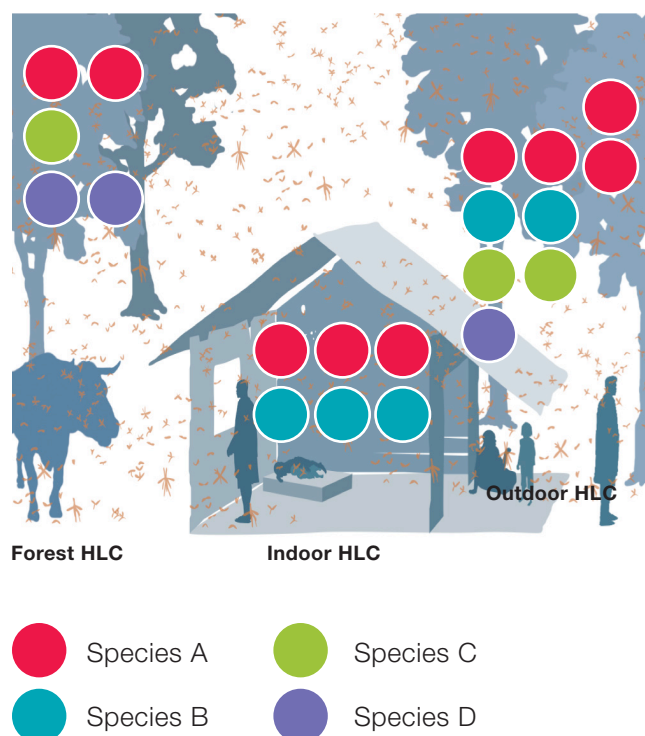
Step 8: Process, collate, and analyze entomological data, [Module 6](#).

Step 9: Interpret results.

1. **Vector occurrence and density:**
 - a. **Indoor HLCs:** captured 2 species identified morphologically, following which molecular methods identified 3 species landing on humans indoors:
 - » Species A (found in large numbers),
 - » Species B (found in large numbers and morphologically identical to Species C), and
 - » Species C (morphologically identified as Species B, determined to be Species C molecularly).

- b. **Outdoor HLCs:** captured 2 species identified morphologically, following which molecular methods identified 3 species landing on humans outdoors:
 - » Species A (found in large numbers),
 - » Species B (found in large numbers and morphologically identical to Species C), and
 - » Species C (morphologically identified as Species B, determined to be Species C molecularly).
- c. **Forest-based HLCs:** captured 3 species landing on humans in forested areas:
 - » Species A (found in smaller numbers),
 - » Species C (morphologically identified as Species B and determined to be Species C molecularly, found in very small numbers),
 - » Species D (found in small numbers).
- d. **Seasonality:** Temporal sampling determined 4 months of peak mosquito density with species-specific peaks.

Figure 4. Representation of vector species found by the various collections. The number of bubbles represents relative density of the different species.



Step 10: Using results described in Step 9 to answer the question, what are the vectors in Area Y?

2. **Species composition and distribution:** Two primary vectors and one secondary vector were found (primary and secondary determined based on relative density) (Table 2). Known vector status of each species collected was derived from the literature.

Table 2. Species collected: vector status and biting location.

Species	Known vector status	Known habitat
A	Primary vector	<ul style="list-style-type: none"> Inside/outside houses (domestic and peridomestic area) Forest
B	Primary vector	<ul style="list-style-type: none"> Inside/outside houses (domestic and peridomestic area) only Larval habitat: rice fields around villages
C	Inconclusive vector status	<ul style="list-style-type: none"> Closely related to B; only distinguished from B molecularly Inside/outside houses (domestic and peridomestic area) Forest
D	Secondary vector	<ul style="list-style-type: none"> Forest and domestic/peridomestic area
E	Not a vector	Forest
F	Secondary vector	Forest

Additional analyses to answer related questions about the relationship between vectors in Area Y, malaria transmission, and rainfall:

3. **Relationship to epidemiological data:** An increase in density of Species A and B populations was found to precede increasing incidence of malaria.
4. **Relationship to rainfall and other potential drivers of transmission:** Occurrence and density of Species A and B populations was found to increase with rains. Species C increases with the rains as well as with rice cultivation periods.

5. **Implications on vector control:** Vector control interventions should target vectors inside and outside houses as well as in forest areas. The appropriate interventions should be selected based on vector and human behavior. Depending on the intervention, the timing of the intervention should precede the rains given the relationship of vector density of Species A, B, and C to rainfall and before rice cultivation for Species C, as well as the relationship between vector density of Species A and B to malaria incidence.

Example C

Step 1: Define your questions, [Module 1](#).

Primary question: When should LLINs and larviciding be deployed in Area Z?

Step 2: Select the relevant indicators to address your question, [Module 2](#).

In [Example C](#), rainfall, temperature, and epidemiological data are considered with entomological data to answer the two sub-questions to the primary question:

Sub-question 1: How does the intervention intersect with vector behavior?

Sub-question 2: What are the drivers of vector populations over time and how do changes in the population over time affect disease transmission?

The two sub-questions above may help answer the primary question about optimal timing for interventions. Intervention deployment should be completed just before the targeted vector populations start to increase.

Since two interventions (LLINs and larviciding) are being considered in this example, the following indicators were selected:

1. **Vector occurrence** to confirm species presence.
2. **Vector density** to examine the relative vector populations and possible species-specific contributions to disease based on density.
3. **Larval habitat occupancy** to confirm which water bodies contain immature vectors.
4. **Larval density** to examine the productivity of larval habitats.
5. **Seasonality** of vectors to identify peaks in species-specific vector populations to examine the association with the seasonality of malaria transmission.

The following data sets were also collated for analysis with the selected indicators above:

1. **Seasonality of transmission** to identify peaks in malaria transmission to examine the association with seasonality of vector populations.
2. **Rainfall and temperature** to evaluate potential climatic drivers of vector populations and malaria transmission.

Note: Susceptibility of local vectors to the active ingredients in LLINs and the larvicide are already available and indicates full susceptibility at present.

Step 3: Determine appropriate sampling methods, [Module 3](#).

Link each of the **indicators** listed in Step 2 to specific **entomological sampling methods** that will generate representative samples of the mosquitoes targeted by the interventions, LLINs and larviciding, and help identify remaining gaps in protection following the deployment of these two interventions.

LLIN-related indicators and sampling methods, with the understanding that LLINs target indoor biting mosquitoes:

1. **Vector occurrence** and **vector density** using either one of two methods:
 - a. **HLCs** to target mosquitoes that bite humans both indoors and outdoors and thus characterize the proportion of mosquitoes targeted by LLINs as well as those that are not targeted by LLINs, OR
 - b. **HLC proxy/substitute**, such as CDC light traps. *Note: before using a proxy method, it is important to understand how well the HLC proxy correlates to HLC collections. In this example, HLCs were selected for use.*
2. **Seasonality of vectors** using **HLCs** (or **proxy**) collections over 1 year to reflect seasonal population changes.

Larviciding-related indicators and sampling methods, with the understanding that larviciding is most effective when coverage is high:

1. **Habitat occupancy and larval density:** use larval dipping to identify the habitats containing larvae and the count of L4 (instar 4) larvae and pupae found, thus indicating habitat productivity.
2. **Vector (larvae) occurrence:** use morphological identification (and molecular identification based on capacity) of larvae reared to adult vectors to identify species-specific larval habitats.

3. **Seasonality of larvae:** to characterize larval sites, presence, and productivity of immature vectors over the course of one year.

Step 4: Select sites, [Module 4](#).

In this example, four villages were selected based on a stratification of Area Z that considered local epidemiology, ecology, and intervention coverage. Four strata were identified and thus one village per stratum was selected for sampling (village = site), with consideration of available resources and capacity. Sampling was conducted once per month over one year.

Step 5: Formulate sampling design, [Module 5](#).

After Steps 1 – 4 were completed, the sampling design below was formulated per site (village):

1. **HLCs:** indoor and outdoor HLCs conducted in 5 sentinel houses for 5 days every month for one year in Area Z. Entomological samples were morphologically identified, and subsequently molecularly identified.
2. **Larval surveys:** potential larval sites were comprehensively surveyed for *Anopheles* larvae for 5 days every month for one year, and all potential habitats were mapped (both those that were positive for *Anopheles* larvae and those that were negative). Larval samples were reared to adults, and morphologically and molecularly identified.
3. **Rainfall and temperature** were documented over the year at the collection sites.
4. **Malaria incidence** data was collected from local health facilities for the same year.

Step 6: Reference decision trees, [Module 7](#).

Baseline trees A: Vector occurrence and density, B: Vector biting behavior, and G: Larval occurrence can support the process of determining appropriate sampling methods, workflow, and design.

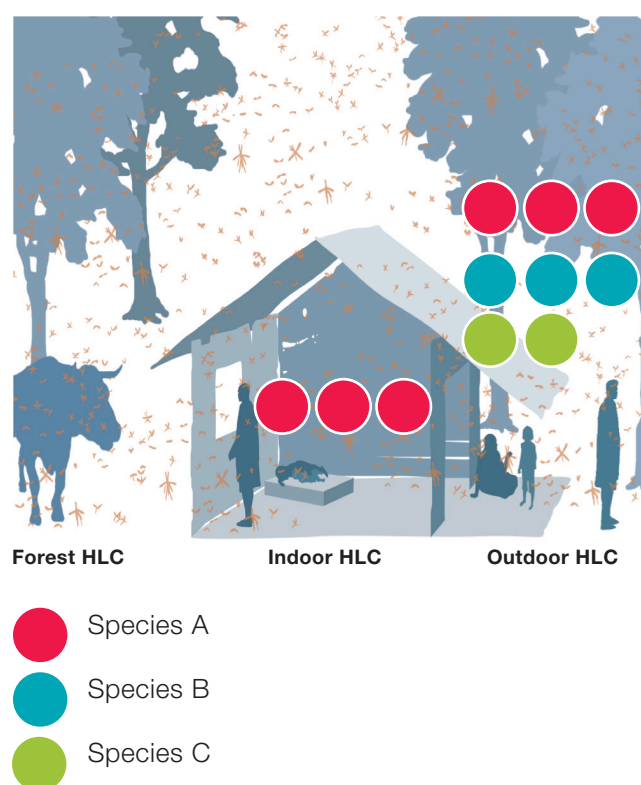
Step 7: Carry out the fieldwork.

Step 8: Process, collate, and analyze entomological data, [Module 6](#).

Step 9: Interpret results.

1. Vector occurrence and density (see [Figure 5](#))
 - a. **Indoor HLCs:**
 - » Species A, found in large numbers, biting indoors throughout the night.
 - b. **Outdoor HLCs:**
 - » Species A found in large numbers, biting outdoors throughout the night.
 - » Species B, found in large numbers, and biting primarily in the evening.
 - » Species C, found in smaller numbers.

Figure 5. Representation of vectors and their location of capture. The number of bubbles represents relative density of the different species



2. **Larval habitat occupancy and larval density**
 - a. **Larval collections:**
 - » Species A and C collected in small rain-filled temporary water bodies and on the edges of larger pools.
 - » Species B collected in more permanent water bodies, including rice fields.
3. **Seasonality of transmission, seasonality of vectors, rainfall, and temperature:**
 - a. Both vector densities and malaria incidence increased following the hot, rainy months.

- b. Malaria incidence slightly decreases following the end of the rains with coincident decreases in Species A and C.

» However, Species B populations remained in permanent larval habitats in irrigated rice fields with coincident ongoing malaria transmission.

Step 10: Use results described in Step 9 to answer the question, when should LLINs and larviciding be deployed in Area Z?

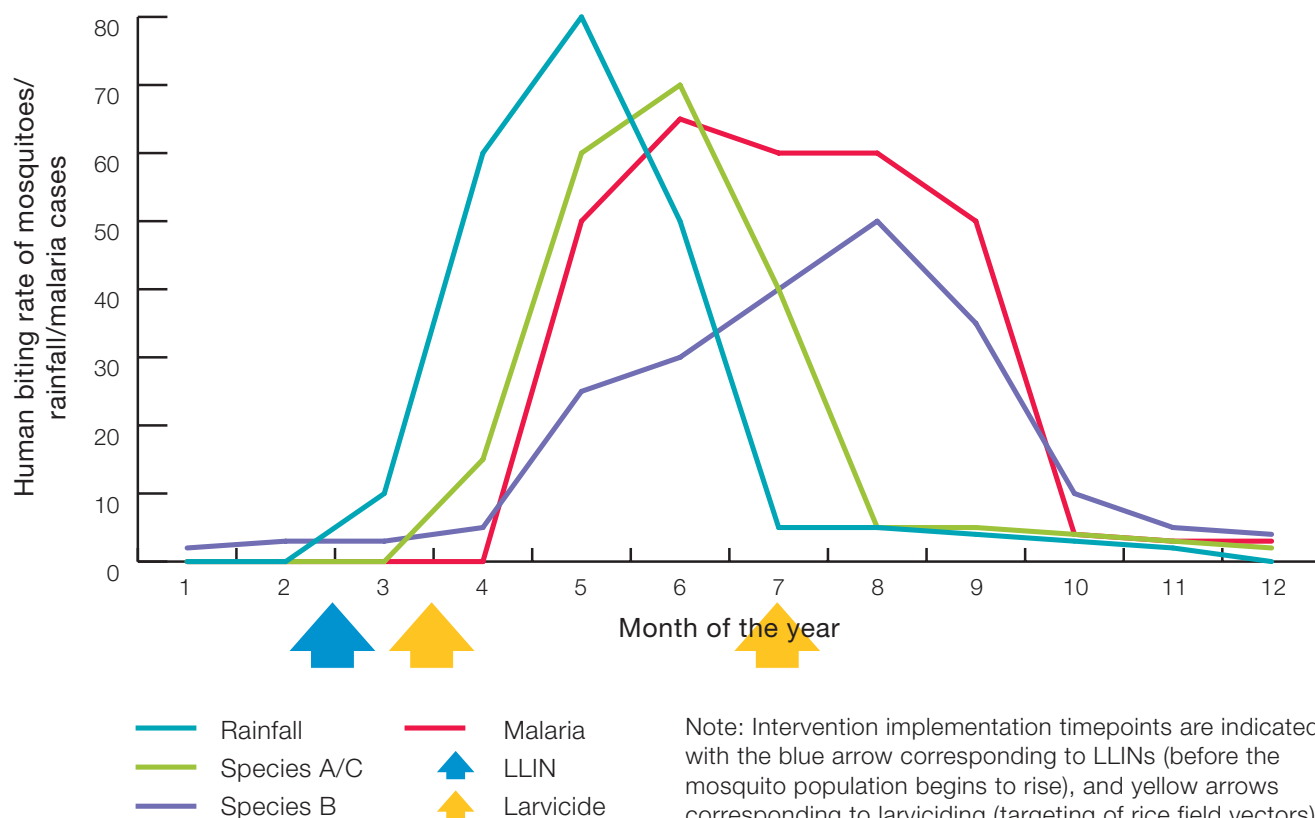
Changes in Species A, B and C densities are associated with changes in malaria incidence; all three vector species should be targeted with a vector control intervention.

- LLINs would primarily target Species A due to its indoor biting behavior. LLINs should be deployed (or intensive hang-up/keep-up campaigns with existing LLINs) before the rains since rainfall is a driver of Species A and C populations.
- Larval habitat surveys identified two types of habitats:

- » Type 1: small temporary bodies of water with Species A and C, which would be difficult to treat with larvicide.
- » Type 2: larger, permanent irrigated rice fields with Species B, which would be treatable with larvicide.
- » Therefore, larviciding may have more impact in permanent rice fields and should commence before the rise of Species B, early in the rainy season. Additional larviciding after the end of the rainy season would help with controlling Species B in these rice fields.

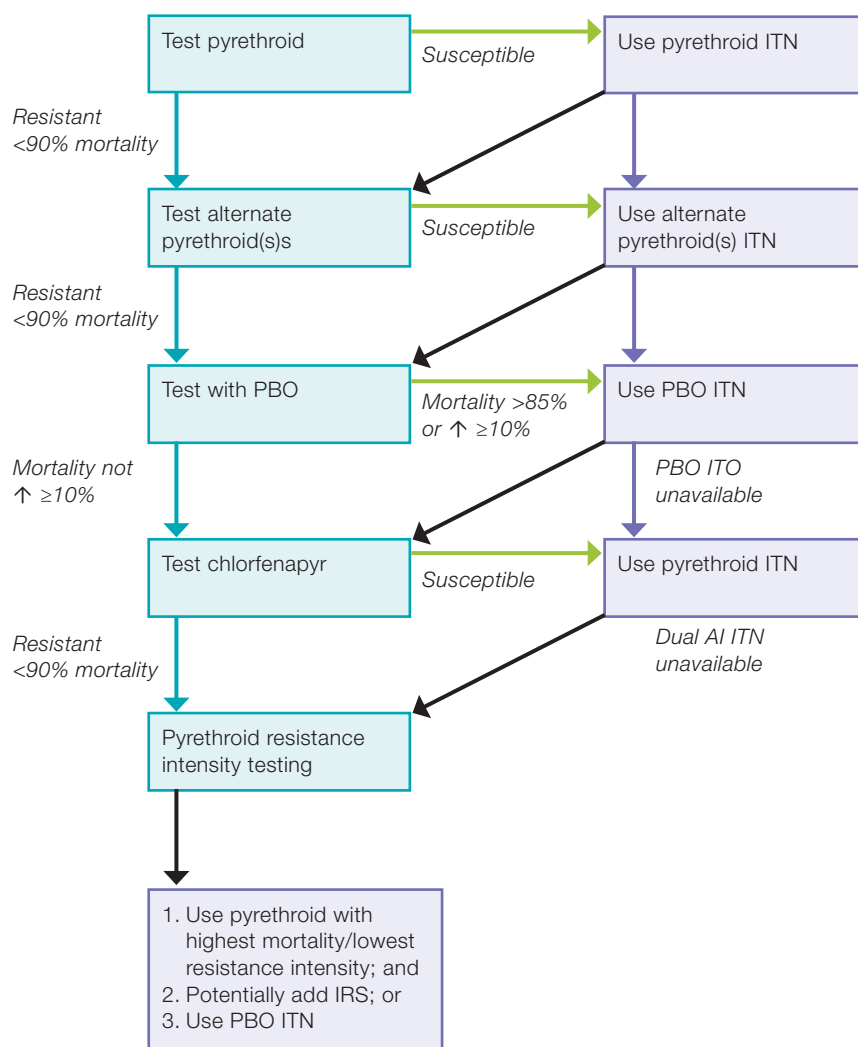
- Gaps in protection that remain after deployment of LLINs and larviciding include:
 - » Biting outdoors by all 3 species, possibly to a lesser extent by Species B if larviciding is effective.
 - » Biting indoors by Species A before people go under their LLINs.
 - » LLINs would primarily impact Species A and not Species B or C.
 - » Larval control likely less effective against Species A and C due to their small temporary larval habitats.

Figure 6. Temporal depiction of rainfall with the seasonal populations of the 3 vectors found



Annex II

Decision Tree for Selecting LLINs Based on Insecticide Resistance Data



Adapted from the President's Malaria Initiative (PMI) Technical Guidelines FY 2020

Annex III

Descriptions of Entomological Sampling Methods and Analytical Techniques

Sampling methods

Human landing catches (HLC)

Human Landing Catches (HLC) sample adult female mosquitoes that are human host seeking. This sampling method contributes to data on vector occurrence and density, biting location (exophagic vs. endophagic), time of biting, analysis of human biting rate (HBR), insecticide resistance and sporozoite rate as described in [Tables 6 and 7 in Module 2](#). HLCs are considered the gold standard for sampling human-biting mosquitoes as they target vectors that are feeding on humans. During an HLC, a person sits at a predetermined location (i.e., inside or outside houses or near high-risk populations in the forest, etc. depending on local transmission dynamics) with their legs exposed to act as bait and attract mosquitoes. As mosquitoes land on the individual, a mouth aspirator is used to collect the specimens before feeding takes place.

HLCs are a technique highly favored by programs and researchers alike because the data is a strong indicator of human-mosquito contact. However, HLCs are labor intensive, expensive, and may expose humans to an increased risk for mosquito-borne diseases. This said, if given malaria prophylaxis as recommended, collectors are often more protected than the general population against malaria.²² Biases in collection can result from insufficiently trained collectors who may not catch mosquitoes at the rate they are present, or because of variable levels of human bait attractiveness to mosquitoes.²³ These limitations can be mitigated by proper training, switching collector locations, and the use of an HLC supervisor who supervises collectors.

Human behavior observations (HBO)

Human behavior observations during HLCs record observations of the number of people present, active or utilizing interventions at the HLC site (usually outdoors or indoors). HLCs can quantify behavioral

interactions between humans and mosquitoes as well as evaluate the protective efficacy of certain interventions and characterize gaps in protection and relative biting risk.²⁴ It is often the HLC collector who documents HBOs or the HLC supervisor. An example HBO data collection form can be found in [Annex IV](#).

Human baited traps (HBT)

Human baited traps (HBT) also sample adult female mosquitoes that are human host seeking. The primary difference between HBTs and HLCs is that usually there is a barrier between the human host/bait and the vector. This sampling method may be used to answer questions about vector species targeting humans, biting location, biting time, HBR, insecticide resistance, and sporozoite rate as described in [Tables 6 and 7 in Module 2](#). There are a variety of HBTs including tent traps (the most common), Ifakara tent trap (ITT), Furvela trap, and odor baited entry trap (OBET), among others. Note these traps may function differently depending on local vector species and should have local data produced that demonstrates local efficacy. See human and animal odor-baited traps below for more ideas on how sampling techniques can be adapted for HBTs. Factors that may affect the use of HBTs include the weight and cost of the tents and the capacity and logistics required to store and transport them..

Indoor resting collections (IRC)

Indoor resting collections (IRC) target the indoor resting behavior of mosquitoes. This method does not capture mosquitoes that do not enter or rest in houses or those that enter and leave before indoor resting collections are conducted. Biases may be introduced into the data based on the type of structure used for collections. For example, if using IRCs to investigate host preference, human houses may have mosquitoes that have fed on humans, and animal shelters may have mosquitoes that have fed on animals. The type of roofing (metal or thatch) may also affect IRC efficacy, along with status of IRS in that structure and insecticide resistance.

Pyrethrum spray catches (PSCs) and aspiration (manual, mechanized backpack, or Prokopack) are commonly used IRC methods. PSCs are conducted

22 Gimnig JE, Walker ED, Otieno P, et al. Incidence of malaria among mosquito collectors conducting human landing catches in Western Kenya. *Am J Trop Med Hyg*. 2013;88(2):301–308.

23 Wong J, Bayoh N, Olang G, et al. Standardizing operational vector sampling techniques for measuring malaria transmission intensity: Evaluation of six mosquito collection methods in Western Kenya. *Malar J*. 2013;12: 143

24 Killeen GF. Characterizing, controlling and eliminating residual malaria transmission. *Malar J*. 2014;13:330

in the early morning before resting mosquitoes leave the houses. Insecticide is used to knock down or kill indoor resting mosquitoes that are then collected on a white sheet. Indoor aspirations do not use insecticide; these aspirations use manual suction or suction devices to collect live mosquitoes resting on the walls.

CDC light trap (CDC-LT)

CDC light traps (CDC-LT) are a suction method of sampling that capture mosquitoes in the vicinity of the battery-operated device. These traps may be used with various baits that include placing them by a sleeping person, using UV light, a carbon-dioxide source, etc. The efficacy of this device can be very variable based on the location and local species bi- nomics. This device is known to function better in- doors with often lower efficacy outdoors depending on the setting. The CDC-LT is the most commonly used HLC-proxy when placed next to a sleeping per- son. Here the capture rates are assumed to reflect those of an HLC as those mosquitoes targeting the sleeping person should be captured by the CDC-LT. Understanding how CDC-LTs function compared to HLCs is important for analysis.

Human odor baited traps (HOBT)

Human odor baited traps (HOBT) exploit human host seeking mosquitoes using synthetic human odors to lure mosquitoes seeking a blood meal. One exam- ple of an HOBT is the Suna trap. This type of trap releases a human-like odor and is often modified to also produce CO₂ to mimic a human. The Suna trap has a vacuum component so that mosquitoes flying toward the source of odor and/or CO₂ are captured in a mesh compartment. HOBTs can be used to gather data on multiple entomological indi- cators including vector occurrence, biting time, and host preference (in conjunction with Animal Baited Traps). Understanding how HOBTs function rela- tive to HLCs in a given location is important when standardizing data for analysis and to limit sampling method introduced biases.

Animal baited traps (ABT)

Similar to HBTs and HOBTs, animal baited traps (ABT) exploit the smell of animals to attract mosqui- toes that feed on the specific animal. When used in conjunction with human baited traps, species-spe- cific zoophily and anthropophily may be determined, as well as overall vector occurrence, density, and composition at a given site. Cows are generally used in ABTs, but other animals such as chickens or goats can be utilized based on local animals present and the question being answered.

Outdoor resting collections (ORC)

Outdoor resting collection (ORC) methods are used to assess outdoor resting behavior of mosquitoes. Mosquitoes need to rest after blood feeding for 1–2 days before oviposition (i.e., laying eggs) so ORCs can also be used to capture blood fed mosquitoes for data on human blood index. Knowledge of the local vector resting behavior is important when using this sampling method since there is such a wide range of possible resting sites present out- doors, which may limit collections and bias the data. Usually an ORC consists of creating a shaded, more humid area for mosquitoes to rest and hide after a blood meal. Examples of ORCs include using aspiration (manual, backpack, or Prokopack), resting pots or boxes, and pit traps.

CO₂ baited trap

Carbon dioxide (CO₂) released by humans and animals attract mosquitoes seeking a blood meal. CO₂ baited traps seek to mimic the CO₂ released by humans (or animals), thereby attracting and trapping host-seeking mosquitoes. For example, a Suna trap or CDC-LT can be equipped to have a CO₂ com- ponent to improve its attractiveness to mosquitoes. Since a CO₂ modified trap may be considered an HLC-proxy (after testing and validation), it can be used to collect samples that are then used to measure multiple entomological indicators such as vector occurrence, vector density, biting behavior, insecticide resistance, and sporozoite rate. Under- standing how CO₂ baited traps function compared to other sampling methods is important for analysis.

Gravid traps

Gravid traps target female mosquitoes in search of a water source to lay their eggs, i.e., ovipositioning females. Several variations on a gravid trap exist, although most have been developed for *Aedes* and have been less effective for *Anopheles*. Recently, there have been developments to specifically sample ovipositioning *Anopheles*.^{25,26} These traps are usually used to look at vector occurrence and preferred larval habitats. Gravid traps can be used to collect data on insecticide susceptibility and sporozoite rate.

25 Harris C, Kihonda J, Lwetoijera D, et al. A simple and effi- cient tool for trapping gravid *Anopheles* at breeding sites. *Parasites Vectors*. 2011;4(125).

26 Dugassa S, Lindh JM, Oyieye F, et al. Development of a gravid trap for collecting live malaria vectors *Anopheles gambiae* s.l. *PLoS ONE*. 2013;8(7).

Interception traps

These traps function by intercepting mosquitoes in flight. Examples include the Window Exit Trap (WET) and the Barrier Screen. WET are designed to trap mosquitoes that attempt to leave a structure through windows or large apertures before the morning.

WETs are usually designed for placement on the outside of windows. They only capture mosquitoes attempting to leave through the aperture where the trap is located. This trap is used to look at mosquitoes that have entered houses, possibly to feed, and then leave before dawn. An example of a sampling strategy to look at vectors that rest and/or feed indoors may include both PSCs and WETs, which would capture mosquitoes that enter houses, feed and leave before dawn (WET) as well as those that feed and rest indoors (PSCs). A WET is usually attached to a window but it can also be attached to other openings (e.g., walls, doors, eaves) to capture exiting mosquitoes based on local vector bionomics as well as house construction. WETs allow for the measurement of vector occurrence and indoor resting behavior and can capture samples for further measurement of sporozoite rate, human blood index, and insecticide resistance.

Barrier screens are a type of interception trap that sample mosquitoes intercepted during flight and rest on the barrier outdoors. Data gleaned from this trap is appropriate to inform flight direction and possibly host seeking. This sampling device may be used to look at flight patterns and infer resting location.²⁷ Blood-fed mosquitoes sampled may be used to look at host preference.

Larval surveys and characterization

Anopheles larval surveys using larval dipping allow for the collection of immature mosquitoes from standing bodies of water. Larval surveys monitor the changes in receptivity related to the occupancy and distribution of positive larval habitats, inform targeting of LSM interventions, and produce larval samples that can be reared to adults for morphological identification (and molecular identification if sufficient capacity exists) and for insecticide resistance testing. Once the presence of larvae is detected, habitats should be characterized based on location, permanence, size, vegetation, predators, etc. to support LSM intervention selection and targeting.

Larval sampling limitations include difficulty in identifying larval samples to species, and the samples captured might not be representative of targeted mosquitoes. (For example, larval sampling may not represent indoor resting vectors if looking at the effect of insecticide resistance on the impact of IRS.) Additionally, larval sampling may miss important vectors present at the site or miss larval sites. (For example, *An. funestus* or *An. dirus* larvae are usually difficult to capture.) Insectaries encounter concomitant challenges when attempting to rear larvae to adults (some species are virtually impossible to rear). When using reared larvae to test for insecticide resistance, care should be taken to use as diverse a number of samples as possible to eliminate the possibility of bias due to using siblings in a sample. In addition, it is often difficult to locate and identify all larval habitats in an area. Local knowledge and community engagement may be particularly useful for this.

Procurement of traps

Awareness of the variations of certain trap types is important to avoid purchasing mosquito traps that are invalid and/or of poor quality. For instance, there are a number of variations of the standardized CDC Light Trap that exist on the market. Although these traps may seem appealing because they are less expensive, they are often unsuitable for scientific entomological activities, as they have not been validated and standardized. Moreover, their quality is often subpar to the original trap version.

Thus, prior to procuring traps, it is important to be informed about the quality and purpose of the traps being considered for purchasing.

Two of the main brands of entomological traps include BioQuip Products, Inc., and The John W. Hock Company. If the cost of traps is a serious concern and you are seeking a valid, less expensive alternative, reach out to experts in the field who may provide guidance.

27 Burkot TR, Russel TL, Reimer LJ, et al. Barrier screens: a method to sample blood-fed and host-seeking exophilic mosquitoes. *Malar J.* 2013;12:49.

Entomological Techniques

Morphological identification using *Anopheles* identification keys

Anopheles identification keys allow a trained technician to match the mosquito sample to the species using known distinct species-specific morphological characteristics. Regional keys are available that represent the varying species complexes by geography.^{28,29,30} Defining morphological features can include colors and banding on antennae and legs, among others. Morphological identification is the most common method used to identify a sample to species. Limitations include the requirement for good training (and retraining), and the inability to distinguish members of sibling species or cryptic species (e.g., *An. gambiae* s.l.). The ability to reference a collection of pinned specimens would greatly enhance the sensitivity and specificity of morphological identification.

Molecular identification

Polymerase chain reaction, or PCR, based diagnostics are molecular biology techniques used to amplify DNA sequences that allow mosquito species to be identified based on species-specific differences in nucleotide sequence and, hence, amplicon length. PCR has a high rate of sensitivity and specificity and is thus a preferred technique for identifying mosquito biodiversity. However, DNA primers for PCR analyses are only available for a limited number of species that include members of the *An. gambiae* complex, *An. funestus* complex, and a few other species.

Sequencing of genomic regions such as the Internal Transcribed Spacer-2 (ITS2) or Cytochrome Oxidase Subunit-1 (CO1) regions, to identify a sample to a species is also possible for species using specific genomic locations if malaria programs and/or research partners have such capacity.

Morphological identification prior to molecular identification enables more efficient molecular processing and greater sensitivity and specificity. Though sequencing can associate specific sequences with a mosquito specimen, the presence of a correctly

morphologically identified sample with the associated sequence is required for identifying the specific specimen to species.

Salivary gland dissections

Salivary gland dissections allow for microscopic observation of sporozoites in freshly killed mosquitoes. Needles are used to dissect the salivary gland from the specimen, thus allowing the sporozoites to be observed under a microscope. The severity of sporozoite infection is graded from 1+ to 4+: 1+ (1–10 sporozoites), 2+ (11–100 sporozoites), 3+ (101–1000), and 4+ (>1000 sporozoites). This technique is labor intensive so training (and retraining) is required. This technique is used to incriminate vectors and to determine sporozoite rates.³¹

Ovary dissections

This technique is used to determine the age structure of the mosquito population differentiating populations based on if they have had a blood meal or not. This technique is labor intensive so training (and retraining) is required. An appropriate sample of freshly caught mosquitoes representing location and time of capture is required to effectively look at age structure.³²

Circumsporozoite (CS) ELISA for sporozoite detection

Circumsporozoite enzyme-linked immunosorbent assay (CS ELISA) is a technique used to detect *Plasmodium* infections in mosquitoes and can thus measure entomological indicators such as sporozoite rate (and thus vector status) and entomological inoculation rate (EIR). For CS ELISA, the head and thorax of the mosquito sample are used to test for the presence of the sporozoite-produced circumsporozoite protein using an ELISA assay.²⁴ Sporozoites can be identified to *Plasmodium* species based on the monoclonal antibody used.

Bloodmeal (BM) ELISA or PCR for host blood detection

The BM-ELISA or PCR is used to determine the source of the mosquito's bloodmeal.²⁴ Here the blood-fed mosquito abdomen is examined using an ELISA or PCR to identify host blood. The technique can be customized to test for human, cow, and other animal sources (both domesticated and wild animals) based on the monoclonal antibody or host

28 Gillies MT, De Meillon B. The *Anophelinae* of Africa south of the Sahara. Johannesburg: Publications of the South African Institute for Medical Research; 1987.

29 Rattanarithikul R, Harrison BA, Harbach RE, et al. Illustrated keys to the mosquitoes of Thailand, Part 4. *Anopheles*. *The Southeast Asian J Trop Med Public Health*. 2006;37 (suppl 2).

30 Wilkerson R, Strickman D, Litwak TR. Illustrated key to the female *Anophelinae* mosquitoes of Central America and Mexico. *J Am Mosquito Contr*. 1990;6: 7–34

31 Benedict MQ. MR4. Methods in *Anopheles* Research. 4th edition. *BEI Resources*. 2014.

32 Kent RJ, Norris DE. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. *Am J Trop Med Hyg*. 2005;73(2): 336–42.

specific PCR primers. Limitations include cross-reactivity between goat and sheep antibodies as well as the inability to determine host when the appropriate species is not included in the test.

PCR for parasite detection

PCR can also be used to detect the presence of the parasite in the mosquito.^{33,34} Usually, the head and thorax is used to limit the DNA detection to infectious sporozoites that leave the abdomen and infect the salivary glands. Since this technique looks at DNA that is found in all stages of the parasite, care should be taken to mention this in any analyses as infection rates (presence of DNA) might not be reflective of infectious rates (presence of infections sporozoites in the salivary glands). The absolute relationship between CS ELISA and *Plasmodium* PCR is not determined at present.

WHO tube bioassay

WHO tube bioassay procedures measure the susceptibility of local vectors to five classes of insecticide, including organochlorines, organophosphates, pyrethroids, carbamates and neonicotinoids.³⁵ The technician should use the test procedures as outlined in the *WHO Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes*.³⁵ Intensity of resistance may also be measured. When presenting results on insecticide resistance, the sampling method used to capture the mosquitoes should be noted, as well as the mosquitoes used (F0 wild caught or F1 progeny) as these may bias results. Controls using colony-reared susceptible mosquitoes should be utilized when available. When susceptible mosquitoes are not available, survival controls are recommended using wild-caught mosquitoes. When insecticide resistance data is presented, the nature of controls used (susceptible mosquitoes, survival controls, or no controls) should be defined.

CDC bottle assay

The CDC bottle assay also looks at frequency and intensity of insecticide resistance.³⁶ When presenting

results on insecticide resistance, the sampling method used to capture the mosquitoes should be noted, as well as the mosquitoes used (F0 wild caught or F1 progeny) as these may bias results. Controls using susceptible mosquitoes should be utilized when available. See above for guidance on controls.

PCR for mechanisms of insecticide resistance

PCR can also be used to evaluate the presence of genes and alleles associated with insecticide resistance, including knockdown resistance (Kdr) target site mutations as associated with resistance to pyrethroids and DDT (both Kdr-East and Kdr-West) and acetylcholinesterase (Ace-1) mutations, which are associated with carbamate and organophosphate resistance in *Anopheles gambiae*. Various species-specific and target site-specific tests are available and should be appropriately conducted based on the questions the tests aim to answer. Controls (resistant, susceptible and heterozygotes) should always be included in the tests, and understanding the interactions between each genotype (e.g., between Kdr-East and Kdr-West) is important when interpreting results. Species identification of samples should always be conducted to ensure that non-targeted species are not included in the analyses.

Cone bioassay

Cone bioassays evaluate the toxicity of insecticide treated surfaces such as LLINs and walls treated with IRS. Here, susceptible mosquitoes are exposed to the treated surface for a certain amount of time to determine effect.³⁷ This method looks at the present and immediate effect of the intervention on susceptible mosquitoes and is usually used to assess residual or temporal bio-efficacy of an intervention's active ingredient.

Further guidance and protocols on laboratory and field entomology methodologies can be accessed through the WHO's Manual on Practical Entomology in Malaria³⁸ (currently being updated) and the Malaria Research and Reference Reagent Resource Center (MR4).³³

33 Snounou G, Singh B. Nested PCR analysis of *Plasmodium* parasites. *Methods Mol Med*. 2002;72:189–203.

34 Echeverry DF, Deason NA, Makuru V, et al. Fast and robust single PCR for *Plasmodium* sporozoite detection in mosquitoes using the cytochrome oxidase I gene. *Malar J*. 2017;16(1):230.

35 WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2nd ed. World Health Organization. Geneva, 2016.

36 WHO. Guidelines for laboratory and field testing of long-lasting insecticidal nets. World Health Organization. Geneva. 2013.

37 WHO. 2013. Guidelines for laboratory and field testing of long-lasting insecticidal nets. World Health Organization. Geneva. 2013.

38 WHO. Manual on practical entomology in malaria. World Health Organization Division of Malaria and Other Parasitic Diseases, Geneva. 1995.

Annex IV

Example Human Behavior Observations Form

Locality _____ District _____ Region _____

Supervisor name _____ Collection Date ____ / ____ / ____ House number _____

Homeowner name _____ GPS coordinates of house: Lat _____ Long _____

Hour of observation	Name of observer	Location of observer (inside/outside)	Number of people at the END of the collection hour:			
			Sleeping		Awake	
			Using a bednet	NOT using a bednet	Using a bednet	NOT using a bednet
6:00–7:00 PM		Inside				
		Outside				
7:00–8:00 PM		Inside				
		Outside				
8:00–9:00 PM		Inside				
		Outside				
9:00–10:00 PM		Inside				
		Outside				
10:00–11:00 PM		Inside				
		Outside				
11:00 PM–12:00 AM		Inside				
		Outside				
12:00–1:00 AM		Inside				
		Outside				
1:00–2:00 AM		Inside				
		Outside				
2:00–3:00 AM		Inside				
		Outside				
3:00–4:00 AM		Inside				
		Outside				
4:00–5:00 AM		Inside				
		Outside				

This human behavior observation (HBO) data collection form examines local people's sleeping and waking patterns and use of LLINs (as applicable) over a 12-hour period. When overlapped with vector behavior and insecticide resistance data, such human behavior data demonstrates where and when people are exposed to mosquito bites, and potential gaps in protection indicating that supplemental tools may be needed.

This form is often completed alongside HLC collections by the supervisor, or by the HLC collectors themselves. Each row corresponds to an HLC collection hour and is filled out at the end of each hour. This form comprises the *minimum* data points required to address when and where humans are exposed and gaps in protection. Thus, note that this form may vary according to additional specificities unique to a program's question.

The following **data dictionary** expands on the type of data collected in each column of the table on the HBO form. Common supplemental variables are also included below.

- **Locality:** Indicate collection locality name.
- **District:** Indicate the full district name in which collection locality is located.
- **Region:** Indicate full region name.
- **Supervisor Name:** Enter full name of supervisor.
- **Collection date:** The date corresponding to when the collection night BEGINS.
- **House number:** Enter the house number corresponding to the HBO collection. Note that this is typically the same house as the house undergoing HLCs.
- **Homeowner name:** Enter the HBO house's homeowner's full name. If multiple homeowners, select one, and stay consistent. Note that this is typically the same house as the house undergoing HLCs.
- **GPS coordinates:** Enter GPS coordinates corresponding to the HLC house. Enter in decimal degree (DD) format.
- **Collection hour:** Distinguishing human behavior hour by hour, throughout the 12-hour night, enables the evaluation of human behavior variation throughout a single night. Thus, each row corresponds to a single observation hour.
- **Name of observer:** Enter the full name of the person making and recording observations. Most often, the observer is the same person doing HLCs.

- **Location of observer:** Indicate whether observations are made indoors or outdoors. Note that "outdoors" is usually considered to be about a 3–5 meter periphery around the HLC/HBO house. Note that these observations may be conducted in other spaces as well (in addition to inside and outside).
- **Number of people at the END of the collection hour:** At the END of the collection hour, count and record (inside and outside):
 - » **Sleeping:** Using a bednet: enter the total number of people sleeping using a bednet. This type of data would be collected when use of bed nets amongst the local population is of interest to the program. Overlaid with vector behavior data (and insecticide resistance status), the program can understand to what extent bednets actually provide protection against mosquito bites within the local population. Thus, here, the observer records the number of people sleeping under a bed net at the end of each collection hour. Note that if bednets are not part of the national strategy, bednets can be removed.
 - » **Sleeping:** NOT using a bednet. Here, the observer records the number of people sleeping without the protection of a bednet (outside of a bednet).
 - » **Awake:** Using a bednet. Enter the number of people that are awake and under a bednet (inside of a bednet).
 - » **Awake:** NOT using a bednet. Enter the number of people awake, and not using a bednet.

Depending on the question posed, **additional variables** can be collected during HBOs. Such variables help gain better understanding of coverage and use of vector control interventions by the local population, as well as overlap of the mosquito behavior targeted by the intervention with the local human behavior.

Additional time: If mosquitoes are found biting either earlier or later than the first or last collection hour (in this example, 6:00 – 7:00 PM, or 5:00 AM – 6:00 AM), which can also be observed by biting rates higher than 0 at the start and end of collections, data collection for both HLCs and HBOs should extend to be able to capture these events.

Annex V

Glossary^{39–43}

Anthropophilic

Description of mosquitoes that show a preference for feeding on humans, even when non-human hosts are available.

Note: A relative term requiring quantification to indicate the extent of preference for anthropophily versus zoophily; usually expressed as the human blood index (proportion of mosquitoes that have fed on humans out of total fed).

Attractive toxic sugar baits (ATSB)⁴⁴

A form of mosquito control based on an "attract and kill" principle where a fruit or flower scent is used as an attractant, sugar solution as a feeding stimulant, and oral toxin to kill the mosquitoes. ATSB solutions are often sprayed on vegetation or suspended in bait stations. ATSBs target both sugar-feeding male and female mosquitoes.

Case detection

One of the activities of surveillance operations, involving a search for malaria cases in a community.

Note: Case detection is a screening process in which the indicator is either the presence of fever or epidemiological attributes such as high-risk situations or groups. Infection detection requires use of a diagnostic test to identify asymptomatic malaria infections.

Case detection, active

Detection by health workers of malaria cases at community and household levels, sometimes in population groups that are considered at high risk. Active case detection can consist of screening for

fever followed by parasitological examination of all febrile patients or as parasitological examination of the target population without prior screening for fever.

Note: Active case detection may be undertaken in response to a confirmed case or cluster of cases, in which a population potentially linked to such cases is screened and tested (referred to as "reactive case detection"), or it may be undertaken in high-risk groups, not prompted by detection of cases (referred to as "proactive case detection").

Case, malaria

Occurrence of malaria infection in a person in whom the presence of malaria parasites in the blood has been confirmed by a diagnostic test.

Note: A suspected malaria case cannot be considered a malaria case until parasitological confirmation. A malaria case can be classified as indigenous, induced, introduced, imported, relapsing, or recrudescent (depending on the origin of infection); and as symptomatic or asymptomatic. In malaria control settings, a "case" is the occurrence of confirmed malaria infection with illness or disease. In settings where malaria is actively being eliminated or has been eliminated, a "case" is the occurrence of any confirmed malaria infection with or without symptoms.

Driver of transmission/transmission driver

Factors that contribute to malaria transmission, such as changes in epidemiology (e.g., increase in malaria cases), entomology (e.g., outdoor vector biting), receptivity (e.g., rainfall that leads to proliferation of larval habitats), importation risk (e.g., population movement), and operational inefficiencies (e.g., stock-outs of ACTs, suboptimal coverage of vector control interventions).

Endectocides⁴⁵

Endectocides have been commonly used in veterinary medicine and increasingly in global health for their antiparasitic activity in humans against onchocerciasis and lymphatic filariasis. Besides their broad antiparasitic activity, some endectocides (e.g.,

39 WHO. WHO malaria terminology. World Health Organization. Geneva. 2016.

40 WHO. A framework for malaria elimination. World Health Organization. Geneva. 2017.

41 WHO. Chapter 5: entomological surveillance and response. In: Malaria surveillance operational manual. World Health Organization. Geneva. 2018.

42 Malaria Elimination Initiative. Malaria High-risk Population Surveillance and Response Toolkit. University of California, San Francisco, Global Health Group. 2017.

43 WHO. Larval source management: a supplementary measure for malaria vector control: an operational manual. World Health Organization. Geneva. 2013.

44 Müller, G.C., Beier, J.C., Traore, S.F. et al. Successful field trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the *Anopheles gambiae* complex in Mali, West Africa. *Malar J.* 2010; 9(210).

45 The Ivermectin Roadmappers. A roadmap for the development of ivermectin as a complementary malaria vector control tool. *AJTMH.* 2020; 102(2s), p. 3-24.

ivermectin), have been shown to kill mosquitoes that feed on treated humans and livestock and are increasingly being evaluated as a malaria vector control tool for large scale public health impact.

Entomology

A branch of zoology that deals with insects.

Focal screening and treatment

Screening and treating a subset of a population or a focus in response to the detection of an infected person.

Focus, active

Focus with ongoing transmission.

Focus, cleared

A focus with no local transmission for more than 3 years.

Focus, malaria

A defined and circumscribed area situated in a currently or formerly malarious area that contains the epidemiological and ecological factors necessary for malaria transmission.

Note: Foci can be classified as active, residual non-active, or cleared.

Focus, residual non-active

A focus where transmission was interrupted recently (less than 3 years ago).

High transmission area

Characterized by an annual parasite incidence of about 450 or more cases per 1,000 population and a *P. falciparum* prevalence rate of $\geq 35\%$.

Importation risk

The frequency of influx of infected individuals or groups and/or infective anopheline mosquitoes.

Indoor residual spraying

Operational procedure and strategy for malaria vector control involving spraying interior surfaces of dwellings with a residual insecticide to kill or repel endophilic mosquitoes.

Integrated vector management

Rational decision-making for optimal use of resources for vector control.

Note: The aim is to improve the efficacy, cost-effectiveness, ecological soundness, and sustainability of vector control activities against vector-borne diseases.

Larval source management

Management of aquatic habitats (water bodies) that are potential habitats for mosquito larvae, in order to prevent development beyond the immature stage.

Note: The four types of LSM are:

1. *Habitat modification: a permanent alteration to the environment, e.g., land reclamation*
2. *Habitat manipulation: a recurrent activity, e.g., flushing of streams*
3. *Larviciding: the regular application of biological or chemical insecticides to water bodies*
4. *Biological control: the introduction of natural predators into water bodies*

Long-lasting insecticidal net

A factory-treated mosquito net made of material into which insecticide is incorporated or bound around the fibers. The net must retain its effective biological activity for at least 20 WHO standard washes under laboratory conditions and 3 years of recommended use under field conditions.

Low transmission area

Areas that have an annual parasite incidence of 100–250 cases per 1,000 population and a prevalence of *P. falciparum*/*P. vivax* of 1–10%. It should be noted that the incidence of cases or infections is a more useful measure in geographical units in which the prevalence is low, given the difficulty of measuring prevalence accurately at low levels.

Malaria elimination

Interruption of local transmission (reduction to zero incidence of indigenous cases) of a specified malaria parasite species in a defined geographical area as a result of deliberate activities. Continued measures to prevent re-establishment of transmission are required.

Note: The certification of malaria elimination in a country will require that local transmission is interrupted for all human malaria parasites.

Malaria eradication

Permanent reduction to zero of the worldwide incidence of infection caused by all human malaria parasite species as a result of deliberate activities. Interventions are no longer required once eradication has been achieved.

Malaria-free

Describes an area in which there is no continuing local mosquito-borne malaria transmission and the

risk for acquiring malaria is limited to infection from introduced cases.

Malaria reintroduction

The occurrence of introduced cases (cases of the first-generation local transmission that are epidemiologically linked to a confirmed imported case) in a country or area where the disease had previously been eliminated.

Note: Malaria reintroduction is different from re-establishment of malaria transmission (see definition).

Mass drug administration

Administration of antimalarial treatment to every member of a defined population or every person living in a defined geographical area (except those for whom the medicine is contraindicated) at approximately the same time and often at repeated intervals.

Note: Mass drug administration is usually performed in order to radically reduce the parasite reservoir of infection and thus reduce transmission in a population.

Mass screening, testing, and treatment

Screening of an entire population for risk factors, testing individuals at risk and treating those with a positive test result.

Moderate transmission area

Areas that have an annual parasite incidence of 250–450 cases per 1000 population and a prevalence of *P. falciparum*/*P. vivax* malaria of 10–35%.

Outbreak

A case or a greater number of cases of locally transmitted infection than would be expected at a particular time and place.

Population at risk

Population living in a geographical area where locally acquired malaria cases have occurred in the past three years.

Prevention of reintroduction

Prevention of the reintroduction of malaria by the occurrence of introduced cases (cases of the first-generation local transmission that are epidemiologically linked to a confirmed imported case) in a country or area where the disease had previously been eliminated.

Note: Malaria reintroduction is different from re-establishment of malaria transmission.

Receptivity

Receptivity of an ecosystem to transmission of malaria.

Note: A receptive ecosystem should have e.g., the presence of competent vectors, a suitable climate, and a susceptible population.

Residual transmission

Transmission that occurs even with good access to and usage of LLINs or well-implemented IRS, as well as in situations where LLIN use or IRS are not practical. A combination of human and vector behaviors is responsible for this transmission, for example, when people reside in or visit forest areas, or do not sleep in protected houses, or when local mosquito vector species exhibit one or more behaviors that allow them to avoid the core interventions.

Sentinel site

A community from which in-depth data is gathered and the resulting analysis is used to inform programs and policies affecting a larger geographic area.

Surveillance, sentinel

Collection and use of data from a random or non-random sample of collecting sites as indicator data for the population as a whole, in order to identify cases of a disease early or to obtain indicative data about trends of a disease or health event that is not malaria specific.

Stratification

Classification of geographical areas or localities according to epidemiological, ecological, social and economic determinants for the purpose of guiding malaria interventions.

Temporal trends

Trends over time, which could be epidemiological, entomological, spatial, and meteorological, including seasonality of transmission (often related to rainfall, temperature).

Vector

In malaria, adult females of any mosquito species in which *Plasmodium* undergoes its sexual cycle (whereby the mosquito is the definitive host of the parasite) to the infective sporozoite stage (completion of extrinsic development), ready for transmission when a vertebrate host is bitten.

Very low transmission area

Areas that have an annual parasite incidence of < 100 cases per 1000 population and a prevalence of *P. falciparum*/*P. vivax* malaria > 0 but < 1%.

Annex VI

Supplementary Vector Control Interventions and WHO Recommendations

Vector control tool	Target mosquito life stage		Target blood feeding preference		Target biting and resting behavior		Highest level of evidence ^{*,46}	WHO policy recommendation (WHO Malaria Vector Control Guidelines 2019) ^{47,48}
	Immature	Adult	Human	Animal	Indoor	Outdoor		
Attract-and-kill not based on sugar							Phase II	No current WHO policy recommendation.
Attractive targeted sugar baits							Phase III underway	No current WHO policy recommendation.
Eave tubes							Phase III underway	No current WHO policy recommendation.
Environmental management							Phase III	No WHO recommendation until evidence is reviewed. According to the WHO Guidelines for Malaria Vector Control (2019), environmental management should, where feasible, be the primary strategy to reduce the availability of larval habitats.
Insecticide-treated clothing							Phase III	Conditional WHO recommendation against deployment as an intervention with public health value due to low certainty-evidence. However, insecticide treated clothing may be beneficial as an intervention to provide personal protection against malaria in specific population groups.
Insecticide-treated hammocks							Phase III	No current WHO policy recommendation.
Insecticide-treated livestock (topical)							Phase III	No current WHO policy recommendation.
Larviciding (aerial)							Phase II	No current WHO recommendation.

 = Yes

*Highest level of evidence determined by published literature of the following study designs: Phase I - laboratory assays to determine the mode of action; Phase II – semi-field, experimental hut, and small-scale field studies; and Phase III – trials measuring the efficacy of the VCT against epidemiological outcomes under optimal conditions.

46 Williams YA, Tusting LS, Hocini S, et al. Expanding the vector control toolbox for malaria elimination: a systematic review of the evidence. *Adv Parasitol*. 2018;99:345-379.

47 WHO. Larval source management: a supplementary measure for malaria vector control: an operational manual. World Health Organization. Geneva. 2013.

48 WHO. Guidelines for malaria vector control. World Health Organization. Geneva, 2019.

Vector control tool	Target mosquito life stage		Target blood feeding preference		Target biting and resting behavior		Highest level of evidence*	WHO policy recommendation (WHO Malaria Vector Control Guidelines 2019)
	Immature	Adult	Human	Animal	Indoor	Outdoor		
Larviciding (manual)							Phase III	Conditional WHO recommendation as a supplemental intervention in areas where high coverage with a core intervention has been achieved, where aquatic habitats are few, fixed, and findable, and where its application is both feasible and cost-effective.
Larvivorous fish							Phase III	No current WHO recommendation due to insufficient evidence.
Livestock endectocides (systemic insecticides)							Phase II	No current WHO policy recommendation.
Mosquito-proofed housing (e.g., window screens)							Phase III	No current WHO policy recommendation. Guidelines under development by the WHO Department of Public Health, Environmental and Social Determinants of Health.
Space spray (aerial)							Phase II	No current WHO policy recommendation for malaria.
Space spray (truck or bike-mounted)							Phase II	Conditional WHO recommendation against deployment based on very low-certainty evidence.
Spatial repellents							Phase III	No WHO recommendation due to very low-certainty evidence.
Topical repellents							Phase III	Conditional WHO recommendation against deployment as an intervention with public health value due to low-certainty evidence. However, topical repellents may be beneficial as an intervention to provide personal protection against malaria in specific population groups.

 = Yes

*Highest level of evidence determined by published literature of the following study designs: Phase I - laboratory assays to determine the mode of action; Phase II - semi-field, experimental hut, and small-scale field studies; and Phase III - trials measuring the efficacy of the VCT against epidemiological outcomes under optimal conditions.

