Cross-sectional survey of pretreatment HIV drug resistance to ARV drugs in infants newly diagnosed with HIV by early infant diagnosis in *[country name], [year of the survey implementation]*

* **Generic protocol version 1.0, 25 June 2024**
* **Nationally representative laboratory-based method**

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# **Resources**

## **Collaborating institutions**

*[Complement as appropriate]*

## **Funding sources**

*[Complement as appropriate]*

# **Abbreviations and acronyms**

|  |  |
| --- | --- |
| ART | antiretroviral therapy |
| ARV | antiretroviral (drug) |
| AZT | zidovudine |
| DTG | dolutegravir |
| INSTI | integrase strand transfer inhibitors |
| NVP | nevirapine |
| PMTCT | mother-to-child transmissions |

# **Definitions**

* **Infants** are defined as children less than 18 months of age.
* **HIV early infant diagnosis** is the testing of HIV-exposed infants to establish timely diagnosis and access to life-saving HIV treatment. Infant diagnosis should be performed using molecular (nucleic acid) technologies in children younger than 18 months.
* **Pretreatment HIV drug resistance** refers to HIV resistance detected among ARV drug-naive people initiating ART or people with previous ARV drug exposure initiating first-line ART. It can result from either transmitted or acquired HIV drug resistance or both. Pretreatment HIV drug resistance may have been transmitted at the time of infection (transmitted drug resistance) or may be acquired from previous ARV drug exposure (such as exposure to ARV drugs for preventing mother-to-child transmission of HIV).

# **Introduction**

HIV drug resistance emerges and is selected when the virus replicates in the presence of antiretroviral (ARV) drugs. HIV resistance to ARV drugs affects the ability of these drugs to block viral replication, negatively affecting the effectiveness of antiretroviral therapy (ART) programmes. HIV drug resistance to ARV drugs decreases the efficacy and options of ART regimens. In addition, it may reduce the prevalence of viral suppression in people with HIV receiving ART, increase the number of new HIV infections and deaths associated with advanced HIV infection, and increase ART program costs (1, 2). Therefore, WHO recommends monitoring HIV resistance to ARV drugs as a key component of a comprehensive and effective HIV response (3, 4).

ART should be initiated urgently in all pregnant and breastfeeding women living with HIV, regardless of when they are diagnosed, including late in pregnancy or postpartum. Reducing maternal viral load is the most effective way to prevent vertical transmission of HIV (5). Currently, WHO recommends using dolutegravir (DTG)-containing ART as a first-line regimen for adults and as a second-line preferred regimen for those receiving a non-DTG-containing ART regimen and who have virologic failure (5).

As part of the prevention of mother-to-child transmissions (PMTCT) programmes, WHO recommends that infants born to mothers with HIV at high risk of acquiring HIV receive daily dual prophylaxis with zidovudine (AZT) and nevirapine (NVP) for the first six weeks of life, regardless of feeding method. High-risk breastfed infants should continue prophylaxis for an additional six weeks (total 12 weeks) using either AZT and NVP or NVP alone. Infants of mothers on ART and breastfeeding should receive six weeks of daily NVP. Infants on replacement feeding should receive four to six weeks of daily NVP or twice-daily AZT (5).

WHO recommends a raltegravir-based ART regimen as the preferred first-line regimen for neonates diagnosed with HIV. WHO recommends using DTG-containing ART for children with ≥3 kg and ≥4 weeks of age (5).

Monitoring pretreatment HIV drug resistance in infants newly diagnosed with HIV is crucial for determining the most effective ART regimens for this population (6). This surveillance approach remains relevant as countries shift to DTG-based ART for adults and children (3). Therefore, WHO recommends monitoring HIV drug resistance to integrase strand transfer inhibitors (INSTIs), along with the reverse-transcriptase and protease regions, as part of the surveillance for HIV resistance to ARV drugs (3).

In *[name of country]*, *[national estimate]* infants are newly diagnosed with HIV annually. According to current national guidance for preventing and treating HIV, for adults and adolescents, the preferred first-line ART scheme in *[country name]* is *[include ART regimen]*, and the standard second-line scheme is *[include ART regimen]*. The preferred first-line scheme for children weighing <20kg is *[include ART regimen],* 20 to 30kg is *[include ART regimen],* and >30kg is *[include ART regimen]*. Infant prophylaxis is prescribed as follow: *[describe infant prophylaxis as per country guidelines]*.

# **Justification**

HIV drug resistance may compromise the efficacy of ARV drugs in reducing HIV-associated HIV incidence and morbidity (1, 2, 7). Even in contexts where ART programs are optimally managed, HIV resistance to ARVs can emerge and be transmitted (8). As the number of people receiving ARVs for HIV prevention or treatment expands, HIV's resistance to ARVs will likely increase (9-11).

To minimise the emergence and spread of HIV drug resistance, WHO recommends that ART and pre-exposure prophylaxis programmes be accompanied by measures to monitor the quality of ART and pre-exposure prophylaxis services, as well as surveillance for HIV resistance to ARV drugs (3, 4), including surveillance of pretreatment HIV drug resistance among infants newly diagnosed with HIV (3, 6).

The results of this survey will be used to inform the national ART guideline and the national action plan to prevent and control HIV resistance to ARV drugs.

# **Objectives**

* To estimate the prevalence of HIV drug resistance by ARV drug among treatment-naïve children less than 18 months of age newly diagnosed with HIV, regardless of PMTCT exposure.
* To estimate the prevalence of HIV drug resistance by ARV drug among treatment-naïve children less than 18 months of age newly diagnosed with HIV, regardless of PMTCT exposure by PMTCT exposure (maternal only, neonatal only, both maternal and neonatal, no or unknown PMTCT exposure).
* To estimate the proportion of treatment-naïve children under 18 months of age newly diagnosed with HIV, categorised by their exposure to PMTCT measures: maternal only, neonatal only, both maternal and neonatal, no PMTCT exposure, and unknown PMTCT exposure.

# **Methodology**

## **Survey design**

A nationally representative cross-sectional survey will be conducted following WHO-recommended methods for the surveillance of pretreatment HIV drug resistance in children newly diagnosed with HIV by early infant diagnosis (6).

Laboratories where EID is performed in the country will contribute eligible case specimens to the survey (**section 7.2**). The survey period will be twelve months, from *[start month]* to *[end month]*. During this period, all eligible case specimens (**section 7.4**) will be stored following WHO recommendations for handling such specimens (12, 13). When the twelve-month period ends, the sample size will be calculated (**section 7.5**). A laboratory’s contribution to the sample will be proportional to the number of eligible case specimens stored.

Selected eligible case specimens will be genotyped (including reverse transcriptase (RT), protease (PR) and integrase (IN) regions of the HIV-1 pol gene) following WHO recommendations (**section 7.7.2**), and the results will be used to estimate the prevalence of HIV resistance to ARV drugs (**section 7.10**).

Demographic information and clinical data, including PMTCT regimen exposure, will be abstracted from laboratory requisition forms, with no participant-level identifying information recorded.

## **Selection of viral load testing laboratories to participate in the survey**

*[Option A (ideal): All EID laboratories in the country will participate in the survey. Below is the list of laboratories and their three-letter unique identifier:*

*\*Include list\*]*

*[Alternative: All EID laboratories in the country will participate in the survey, except for those that handle <10% of total EID tests in the country. Below is the list of laboratories participating in the survey and their unique three-letter identifier. Also included is the list of laboratories that will not participate in the survey:*

*\*Include list\*]*

## **Survey period**

The survey period will be twelve months, from *[start month]* to *[end month]*. All eligible case specimens (**section 7.4**) will be handled and stored according to WHO recommendations during this period (12, 13).

## **Selection of eligible case specimens**

* + 1. **Specimen eligibility criteria**

An eligible case specimen for the survey must meet inclusion criteria and not meet exclusion criteria.

* + - 1. **Inclusion criteria**
* The child is less than 18 months of age;
* The specimen tested HIV-positive by molecular (nucleic acid) technologies
* The remnant specimen is sufficient in quantity for the HIV drug resistance test (at least *[the minimum volume of plasma or number of DBS required by the laboratory that will perform the HIV genotyping tests]*);
* The remnant specimen can be linked to the minimum information needed for the survey (**section 7.9**);
* If more than one specimen is available for the same child, the specimen that tested positive first by molecular (nucleic acid) technologies should be used.
  + - 1. **Exclusion criteria**
* The child is 18 months of age or older;
* The child is receiving three or more ARV drugs for the purpose of HIV treatment (rather than prophylaxis to prevent HIV infection) at the time of the blood draw.

## **Sample size calculation and sampling procedures**

The sample size recommended by WHO (**Annexe 1**) for this survey for a country is 500 in total. A laboratory’s contribution will be proportional to the number of eligible case specimens available per laboratory (6). If there is only one EID laboratory in the country, all case specimens will be sampled from that laboratory. If there is more than one EID laboratory in the country, the overall sample size will be distributed across these laboratories in a manner proportional to the number of specimens available per size.

To determine the appropriate distribution of sample sizes, a list of all laboratories participating in the survey will be compiled (**section 7.2**). Then, the number of eligible case specimens from each laboratory will be recorded. Eligible case specimens are defined as the remnant EID case specimens fulfilling the eligibility criteria (**section 7.4**) collected from each laboratory during the target 12-month survey period. The sample size for each laboratory will be proportionate to its contribution of eligible case specimens (6). For example, if 50% of the total EID case specimens are from a particular laboratory, then 50% of the survey sample size will be allocated to that laboratory. The sample sizes will be rounded to the nearest whole number as necessary.

Once laboratory sample sizes have been determined, each laboratory will randomly select the eligible case specimens through systematic random sampling (**see Annexe 2**). No case specimen will be sampled more than once.

If this sample size exceeds the number of eligible case specimens in the country, a census of all available case specimens will be performed (6).

## **Survey ID**

Eligible case specimens selected to be tested will be identified using the WHO-recommended survey ID, which is composed of the following five elements delimited by a hyphen (-):

* Country abbreviation: The International Organization for Standardization’s standard three-letter abbreviation:[[1]](#footnote-1) *[three-letter ISO code]* for *[country name]*
* Survey type: INF (infant survey)
* Year: The year when the first specimen was collected: *[year]*
* Laboratory ID: Three-letter code identifying the participating EID laboratory (**section 7.2**)
* A four-digit unique number: That is, a consecutive unique number assigned to an eligible case specimen at the survey site.

For example, if the laboratory University Lab *[change this example with the name of an EID lab participating in the survey]* is selected to participate in the survey to be conducted in the Bahamas *[change this example for the name of the country]* in 2024 *[change to the year the survey will start]*, the survey ID for the first eligible case specimen selected would be BHS-INF-2024-UNI-0001 *[change this example accordingly]*.

## **Laboratory procedures**

### **Handling of eligible case specimens**

The specimens for this survey should have been collected and handled following WHO-recommended procedures for surveillance of HIV resistance to ARV drugs (12, 13). Eligible case specimens selected for the survey will be sent to the *[name of HIVDR testing laboratory]* in a cold chain using dry ice and avoiding thawing of specimens (12, 13). The shipment of specimens (packaging, classification and labelling) will be carried out according to the regulations of the International Air Transport Association. Import and export permits will be obtained before sending the samples.

### **HIV drug resistance testing**

WHO strongly recommends the use of WHO-designated laboratories for HIVDR surveillance.[[2]](#footnote-2) Therefore, eligible case specimens will be tested for HIV drug resistance. HIV drug resistance testing will follow WHO recommendations (12) at *[name of laboratory]*, a laboratory designated by WHO for the surveillance of HIV drug resistance. Drug resistance genotyping should include sequencing of the integrase, reverse-transcriptase and protease regions of the HIV-1 *pol* gene (12). The sequences will be identified using the survey ID (**section 7.6**).

The sequences will be assembled using ReCall (British Columbia Centre for Excellence in HIV/AIDS, BCCfE, Vancouver, BC) (14). The sequence quality control will be done using the WHO/BCCfE HIVDR quality control tool: <https://recall.bccfe.ca/who_qc/> (15). If pairs or groups of viral sequences with a small genetic difference (<0.5%) are identified, the HIV genotyping process will be repeated to rule out contamination errors.

## **Management of clinical and demographic data**

Required demographic and clinical data (**section 7.9**) will be obtained from electronic laboratory databases *[Alternatively: manually extracted from EID requisition forms]. [If necessary: [if necessary, the information will be supplemented using records from the clinics from which the specimens were obtained.]* Demographic and clinical data will be identified using the survey ID (**section 7.6**). A survey coordinator will evaluate the quality of the data. Discrepancies, such as inconsistent or out-of-range responses and missing data, will be reported and corrected as appropriate.

## **List of variables to be collected**

### **Required specimen-level information**

1. Specimen survey ID (**section 7.6**)
2. Infant’s clinic ID[[3]](#footnote-3)
3. Date of birth (DD/MM/YYYY)
4. Age of child in months on the date of specimen collection
5. Date of specimen collection (DD/MM/YYYY)
6. Mother exposed to ARV drug(s) during pregnancy and/or breastfeeding? (This includes exposure for the purpose of treatment for her health or for PMTCT): (yes/no/unknown);
   * If yes, include the name of each ARV drug
7. The child received postnatal ARV drug prophylaxis? (yes/no/unknown);
   * If yes, include the name of each ARV drug

### **Required laboratory-level information**

* EID laboratory name
* EID laboratory ID (**section 7.2**)
* The total number of eligible case specimens available at the laboratory during the defined 12-month period ***Note:*** *This variable is essential to perform statistical analysis adjusted with statistical weights according to the survey design.*
* The total number of eligible case specimens sampled at the laboratory. ***Note:*** *This variable is essential to perform statistical analysis adjusted with statistical weights according to the survey design.*
* Total number of eligible case specimens sampled that were successfully genotyped.

## **Statistical analysis**

The WHO/BCCfE HIVDR quality control tool (15) will be used to assess the quality of the HIV sequences. If pairs or groups of viral sequences with a genetic difference <0.5%, with no apparent epidemiological link, are identified, only one sequence from that group will be included in the data analysis.

HIV resistance to ARV drugs will be predicted using the Stanford University HIVdb (16, 17). The virus will be considered resistant when the HIVdb assigns a score ≥15 to a given ARV drug. HIV resistance disaggregated by ARV drugs will be reported. The version of the Stanford algorithm used in the prediction of HIVDR will be recorded and reported.

Statistical analysis will be performed according to the WHO guidelines (6) using STATA (StataCorp, College Station, TX, USA). The analysis will account for genotyping success rate and will be adjusted for a finite population. Proportions and their 95% confidence intervals will be estimated.

## **Ethical considerations**

*[Select the appropriate option and edit accordingly:*

*Option A: This protocol has been evaluated and approved by the ethics committee [insert name of committee]. Since HIV genotyping testing will be performed on deidentified remnant EID specimens, informed consent will not be required.*

*Option B: This protocol describes a surveillance activity for HIV resistance to ARV drugs. Therefore, local regulations have approved this protocol as a surveillance activity, and informed consent will not be required.]*

Staff participating in the survey will be trained on the purpose and appropriate procedures for storing and selecting eligible case specimens for the HIV drug resistance survey. The training will address maintaining the confidentiality of the patient's data whose specimens will be included in the survey.

### **Risk to participants**

This study protocol poses minimal risk. Specimens are collected for routine diagnosis and clinical monitoring in compliance with the national standard of care, and demographic and clinical data are abstracted from laboratory requisition forms.

### **Benefits to participants**

The survey outcomes will inform treatment guidelines for population-level benefits.

*[Countries wishing to return survey results to patient files to guide future treatment decisions may choose to do so. If so, please add the following:*

*HIVDR testing results will be provided to the attending clinician and added to the patient’s clinical record. The findings could assist clinical management, but the time taken for testing will depend on specimen processing and shipment. Therefore, initiation of ART will not be delayed while waiting for an HIVDR test result from a survey specimen.*

*Patient management will follow national guidelines. Expert clinicians in the country will interpret HIVDR testing results with support from WHO.]*

# **Dissemination of survey outcomes**

The survey findings will be included in a report. It will be discussed within the Ministry of Health and used to develop/update the national action plan to prevent and control HIV drug resistance and the national ART guideline. The report will also be shared with different Ministry of Health entities as appropriate and with partners in the national HIV response.

The survey findings will be published in scientific journals as deemed appropriate by the Ministry of Health. In addition, survey data (deidentified demographic, clinical and laboratory data) will be shared with WHO for inclusion in regional and global analyses.

# **References**

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4. Global Action Plan on HIV drug resistance 2017–2021. Geneva: World Health Organization; 2017. p. 40.

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10. Cambiano V, Bertagnolio S, Jordan MR, Pillay D, Perriens JH, Venter F, et al. Predicted levels of HIV drug resistance: potential impact of expanding diagnosis, retention, and eligibility criteria for antiretroviral therapy initiation. AIDS (London, England). 2014;28 Suppl 1:S15-23.

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12. WHO HIVResNet HIV drug resistance laboratory operational framework. Second ed. Geneva: World Health Organization; 2020. p. 82.

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15. WHO HIVDR QC Tool. In: Woods CK, Harrigan PR, editors. <https://pssm.cfenet.ubc.ca/who_qc>.

16. Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. Nucleic acids research. 2003;31(1):298-303.

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## **Annexe 1:** Sample size calculation

Table A.1.1 displays the assumptions used for the sample size calculation. The expected proportion of EID case specimens with PMTCT exposure is assumed to be 80%. As a result, it is assumed that the proportion of EID case specimens with no or unknown PMTCT exposure is 20% (). A maximum CI half-width of ±11% () is suggested as an appropriate compromise between feasibility and precision; this limit is reached when the known versus no or unknown PMTCT exposure breakdown is 80%/20%, although the CI is expected to be narrower (more precise) when the breakdown is closer to 50%/50%.

The assumption for the prevalence of HIVDR 50% (). This is the most conservative choice because it requires the largest sample size. No design effect is required because a stratified design (population stratified on laboratory) is being used in which the size of each population (number of case specimens per laboratory) is known prior to sampling. In addition, 20% of genotypes is assumed to be unsuccessful (). The general form for the sample size is therefore:

Under the assumptions outlined in Table A.1.1, WHO-recommended sample size is 500 in total. If this sample size exceeds the number of eligible case specimens in the country, a census of all available eligible case specimens will be performed.

**Table A.1.1 Assumptions for the sample size calculation**

|  |  |
| --- | --- |
| **Assumptions** | **Proposed values** |
| Expected prevalence of HIV drug resistance | 50% |
| Expected genotyping failure rate | 20% |
| Expected proportion of EID case specimens with known PMTCT exposure | 80% |
| Desired CI half-width | ±5% |
| Maximum desired CI half-width | ±11% |

## **Annexe 2:** Systematic sampling

Systematic sampling will be carried out as follows in each EID laboratory (**example in Table A2.1)**:

1. A **sampling frame** will be constructed: List all eligible case specimens during the survey period *[at the end of the 12-month survey period, list the eligible case specimens and assign a correlative number starting at 1]*
2. The **sampling interval** will be calculated by dividing the total number of eligible case specimens by the number of eligible case specimens to be sampled. The sampling interval will be rounded so as not to include decimal places.
3. A random number will then be selected to initiate systematic sampling. A random number between 1 and the sampling interval *[rounded sampling interval without decimals]* will be generated using the random number generator at <https://openepi.com/Random/Random.htm>.
4. The first eligible case specimen to be selected will be the one that corresponds to the random number selected for each sampling frame.
5. The sum of the initial random number and the sampling interval will correspond to the second eligible case specimen to be selected.
6. The sum of the second eligible case specimen selected and the sampling interval will correspond to the third eligible case specimen to be selected and so on.

**Table A2.1.** Example of a sampling frame and selection of eligible case specimens

|  |  |  |  |
| --- | --- | --- | --- |
| **Correlative number** | **Sample ID** | **Selection** | **Selected eligible case specimen** |
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  | 4† (first sample selected) | Yes |
| 5 |  |  |  |
| 6 |  |  |  |
| 7 |  |  |  |
| 8 |  |  |  |
| 9 |  | 4+5\*=9 | Yes |
| 10 |  |  |  |
| 11 |  |  |  |
| 12 |  |  |  |
| 13 |  |  |  |
| 14 |  | 9+5=14 | Yes |
| 15 |  |  |  |
| 16 |  |  |  |
| 17 |  |  |  |
| 18 |  |  |  |
| 19 |  | 14+5=19 | Yes |
| 20 |  |  |  |
| 21 |  |  |  |
| 22 |  |  |  |
| 23 |  |  |  |
| 24 |  | 19+5=24 | Yes |
| 25 |  |  |  |
| 26 |  |  |  |
| 27 |  |  |  |
| 28 |  |  |  |
| 29 |  | 24+5=29 | Yes |
| 30 |  |  |  |
| 31 |  |  |  |
| 32 |  |  |  |
| 33 |  |  |  |
| 34 |  | 29+5=34 | Yes |
| 35 |  |  |  |
| 36 |  |  |  |
| 37 |  |  |  |
| 38 |  |  |  |
| 39 |  | 34+5=39 | Yes |
| 40 |  |  |  |
| 41 |  |  |  |
| 42 |  |  |  |
| 43 |  |  |  |
| 44 |  | 39+5=44 | Yes |
| 45 |  |  |  |
| 46 |  |  |  |
| 47 |  |  |  |
| 48 |  |  |  |
| 49 |  | 44+5=49 | Yes |
| 50 |  |  |  |
| Total number of eligible case specimens stored during the survey period at X laboratory: | | | 50 |
| Sample size: | | | 10 |
| \*Sampling interval: | | | 50/10 =5 |
| †Random start generated using <https://openepi.com/Random/Random.htm>: | | | 4 |

## **Annexe 4:** Timeline

*[to be adjusted]*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Activity** | **Month 1** | **Month 2** | **Month 3** | **Month 4** | **Month 5** | **Month 6** | **Month 7** | **Month 8** | **Month 9** | **Month 10** | **Month 11** | **Month 12** | **Month 13** | **Month 14** | **Month 15** | **Month 16** | **Month 17** | **Month 18** | **Month 19** | **Month 20** |
| **Planning phase** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Protocol development | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Protocol approval |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Training |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Import and export permits |  |  |  |  |  |  |  |  |  |  |  |  |  | X | X |  |  |  |  |  |
| **Implementation phase** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Selection and storage of eligible case specimens |  |  |  | X | X | X | X | X | X | X | X | X | X | X | X |  |  |  |  |  |
| Selection of eligible case specimens |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |  |  |  |  |
| Shipment of eligible case specimens for HIV drug resistance testing |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |  |  |  |  |
| HIV drug resistance testing |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X | X |  |  |
| Data analysis and draft report |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |  |
| Dissemination of survey outcomes |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |

1. ISO 3166 country codes: <https://www.iso.org/obp/ui/#search/code/> [↑](#footnote-ref-1)
2. The WHO country office will help connect with a WHO-designated laboratory in the region that can perform HIVDR testing for the survey. *[If not applicable, please remove this footnote]* [↑](#footnote-ref-2)
3. This variable is not used in analysis; however, a code linking the assigned survey identification code and the infant’s clinic ID should be maintained at the EID laboratory to facilitate quality assurance and return of results to participants’ medical records, if desired. [↑](#footnote-ref-3)