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DEFINITIONS

Bayesian inference: method of statistical inference that allows combination of prior information on a population parameter with evidence from information contained in a sample to guide the inference.

Congenital abnormalities: birth defects, structural or functional anomalies that occur prior to birth and can be identified prenatally, at birth, or sometimes later in infancy.

Consortium: the collaborative research group of Partners formed to undertake the Project.

Cross-reactivity: the reaction between an antibody and an antigen that differs from the immunogen.

Partner: each beneficiary who is a signing part of the Grant Agreement under which the Project is financed.

Project: ZIKAction Project, financed within the H2020 Research and Innovation Programme of the European Union, under Grant Agreement No. 734857.

Sensitivity: the ability of a diagnostic test to correctly identify those with the disease.

Specificity: the ability of a diagnostic test to correctly identify those without the disease.

Stakeholder: a person, group or organization that has an interest in the Project.

Vertical Transmission: passage of disease causing agent from mother to child in the period immediately before and after birth.

ABBREVIATIONS

CHIKV: Chikungunya virus
DENV: Dengue virus
GA: gestational age
IgM: immunoglobulin M
IgG: immunoglobulin G
MI: maternal infection
PCR: polymerase chain reaction
RCT: randomized clinical trial

SAP:	statistical analysis plan
VI:	vertical infection
VTR:	vertical transmission rate
WP:	work package
ZIKV:	Zika virus

1. Introduction

1.1 The role of Statistical analysis plans (SAPs)

SAPs are commonly used to prevent selective use of data and data dredging by requiring the statistical data analysis to be specified in advance of data collection. SAPs have become a fixed feature of randomized clinical trials (RCTs). Because RCTs are specifically designed and powered around a single hypothesis, with exposure variables and a single primary outcome that are readily defined, every statistical analysis can be specified in detail in advance. Pre-specified SAPs are widely regarded as being desirable for observational studies as well, but there is no generally agreed format. It is recognized, however, that there must be room for an exploratory hypothesis-forming element [1] rather than rigid adherence to testing pre-specified hypotheses. This is especially relevant for vertically acquired ZIKA infection, whose clinical spectrum remains to be defined, and where the interpretation of laboratory measures of exposure is still very uncertain.

1.2 Outline of this document

We begin by defining the three sets of objectives of ZIKA-VT (Section 2). We then explain its relationship to ZIKA-PED and ZIKA-VID because the data collection processes for all these programs are inter-related (Section 3). In Section 4 we set out the properties that the SAP is designed to have in order to meet the requirements of ZIKA-SYN, which is to allow a joint statistical analysis of ZIKA-VT alongside data from other prospective studies of vertical transmission.

Section 5 gives a background on the issues arising in estimating the vertical transmission rate. We then set out the SAPs for each of the areas of study in turn. Basic analyses of vertical transmission and clinical outcomes appear in Section 6, and methods for the full analysis in Section 7. Section 8 covers population epidemiology; analyses of clinical outcomes in women infected in pregnancy are found in Section 9.

Due to the nature of arbovirus transmission, and issues with the serological and virological tests, the ZIKA epidemic raises problems that require extensions to standard statistical methods. In order to keep the overall narrative clear, the more technical material appears in Appendices.

At the same time, certain aspects of the ZIKA-VT protocol have not yet been finalized: in particular, the choice of diagnostic tests. ZIKV diagnostics is a rapidly moving area of research, and we expect there will be changes during the period of study in which tests will be used in field work. Further tests to identify maternal, congenital and post-natal pediatric infection will be carried out on stored samples prior to the “final” analysis. Inevitably, therefore, important elements of the SAP are somewhat generic.

1.3 Levels of analysis

The vertical transmission component of ZIKA-VT is complex observational study, which can be analysed at many different levels. A "face value" analysis, for example, might tabulate the vertical transmission or congenital malformation outcomes by laboratory findings during pregnancy, categorized as "Any laboratory evidence of maternal infection" *vs.* "No evidence of maternal infection". A further elaboration would be to break the lab findings down by trimester of first +ve test.

A slightly more sophisticated analysis, instead of tabulating trimester of the first +ve test, would combine the data on the dates of the tests with information on test persistence, using a pre-specified set of rules, to derive a variable that can be interpreted as "most likely trimester of maternal infection". A still more detailed analysis would additionally take into account surveillance information on what viruses were circulating and at that time, as well as information on test sensitivity, specificity and cross-reactivity. Surveillance data may carry more information about the timing of infection than the tests results. This information would be used to assign a probability that maternal infection took place in the 1st, 2nd, or 3rd trimester. Note that the more "basic" analyses are not simply "initial" analyses: they are needed at every stage to maintain transparency, and serve as essential preliminaries to the more sophisticated analyses that will be needed.

The extra value contributed by the more sophisticated analysis depends primarily on the accuracy of diagnostic tests and the quality of information on test persistence. Calculations in *section 7.6* show that, given the accuracy of currently available diagnostic tests, quite complex analyses may be required to avoid very substantial biases in estimates of the single most important target parameter: the vertical transmission rate.

For this reason, and where appropriate, the SAP will specify both "basic" tables, as well as describing the more sophisticated analyses that will, certainly at our present state of knowledge, be necessary.

2. Objectives of ZIKA-VT

There are three sets of objectives.

(1). The main objectives of ZIKA-VT involve assessment of vertical transmission rate, and the sequelae of vertical transmission for the fetus and the live-born child.

The follow-up of many thousands of women through pregnancy creates an opportunity to answer two further sets of questions relating to: (2) the population epidemiology of ZIKV, DENV and CHIKV and (3) natural history of these infections in the infected mother.

2.1 Objectives relating to vertical transmission

There are two distinct questions relating to vertical transmission answered by the studies in ZIKA-VT and additional data from ZIKA-PED. The first concerns the risk of in utero transmission of ZIKV among

infected mothers, while the second addresses the risk of adverse outcomes of maternal infection in the fetus, newborn and developing child.

In more detail, the objectives are therefore to estimate the role of gestational age (GA) at maternal infection (ZIKV, CHIKV, or DENV) and other risk factors in determining:

(a) The vertical transmission rate (VTR)
(b) *Outcome of pregnancy following maternal infection*: Fetal development, abnormalities on ultrasound, and outcomes of pregnancy (live-birth, stillbirth, miscarriage); GA at delivery; birthweight; anthropometrics including small for GA; cranial disproportion, and
(c) *Pediatric outcomes following maternal infection*: Frequency, type, and severity of adverse outcomes (for example: growth, neurological, neurodevelopmental, ophthalmological, and auditory outcomes) up to 2-4 years of age:

In addition, the impact of maternal infection on both outcomes of pregnancy and pediatric outcomes must be studied: (i) in the presence of *in utero* transmission, (ii) in the absence of *in utero* transmission

The key risk factors of scientific interest for VTR, and for type and severity of pregnancy and pediatric outcomes, are: occurrence of maternal symptoms such as rash; co-infection with other arboviruses during pregnancy; previous infection with the same or different arboviruses, other co-infections. We will also recognize the possibility that VTR and risk of severe outcomes may vary between the collaborating centers, even when these risk factors are taken into account. Variables reflecting socio-economic class or level of education will be treated as potential confounding variables as they may be correlated with both outcomes and exposure to ZIKV.

The same analysis plan will be applied to ZIKV, CHIKV and DENV.

2.2 Objectives relating to the population epidemiology of ZIKV, DENV and CHIKV

(i) The incidence and cumulative incidence of arbovirus infections in areas contributing data; the prevalence of co-infections; impact of previous infections
(ii) The proportion of infections that are symptomatic, and the impact of previous infection with the same or other arboviruses.
(iii) Alignment with national or regional surveillance data, to obtain population estimates of national or regional incidence of ZIKV, DENV, CHIKV whether symptomatic or not.

2.3 Objectives relating to outcomes for the infected mother.

(i) Spectrum of clinical sequelae following symptomatic and asymptomatic infections, and time from infection to the appearance of sequelae.
(ii) Impact of pregnancy on symptoms at presentation, and on spectrum and timing of sequelae
(iii) Impact of cofactors on symptoms at presentation, and on spectrum and timing of sequelae, including co-infection or previous infection with the same or different arboviruses.

3. Relation to ZIKA-PED, ZIKA-VID and ZIKA-SYN

3.1 Outcomes of postnatal pediatric infection: ZIKA-PED.

Follow-up of postnatal infections, both primary and secondary, are within the remit of ZIKA-PED. However, postnatal infection will also be observed in ZIKA-VT. The statistical analysis of clinical symptoms and outcomes following postnatal infection seen in ZIKA-VT will be considered as part of the main ZIKA-PED analysis. However, estimation of post-natal ZIKV incidence rates in the control groups, part of ZIKA-PED, may be used to assist in the analysis of vertical transmission rates (see below and *Appendix 5*).

By the same token, after the two-year follow-up specified in ZIKA-VT, further follow-up of the three ZIKA-VT birth cohorts – Vertically Infected (VI), Maternal Infection no VI (MI – No VI), and No Maternal Infection (No MI) – will be undertaken within ZIKA-PED. However, to answer the scientific questions about outcomes of vertical infection, the statistical analysis of outcomes of vertical transmission after 2 years will be regarded as being within the remit of the ZIKA-VT statistical analysis plan. The ZIKA-PED (prospective) SAP will focus on outcomes of post-natally acquired ZIKV, which will be observed in the MI-No VI and No MI cohorts.

3.2 Evaluation of diagnostic methods: ZIKA-VID.

One of the tasks of ZIKA-VID is to evaluate the diagnostic tests to be used in ZIKA-VT. This comprises both tests undertaken to establish women's infection before and during pregnancy, and tests to establish fetal infection. The evaluation of virological and serological tests in this context consists in estimating their sensitivity, specificity, cross-reactivity (a particular cause of lack of specificity), and persistence. Persistence refers to the duration of a positive response – for example a positive PCR or IgM response – or the duration of a rising IgG response. The ZIKA-VID evaluation will look at the analytic sensitivity and specificity of the tests, but also their diagnostic sensitivity and specificity, which must take into account background incidence. The evaluation will be based on a combination of published literature, specific laboratory studies undertaken within ZIKAction, and information about tests gathered in the course of ZIKA-VT. There is a rapidly developing literature on properties of tests that will be kept under constant review as it bears on the choice of diagnostic algorithms that will be used both in ZIKA-VT field work, and choice of tests and algorithms that will be used in a final data analysis. An Expert Group on ZIKA Diagnostics will be formed to take decisions on both choice of testing methods, and interpretation of test results (see *Appendix 1*).

Although the evaluation of the persistence of PCR and IgM tests properly falls within the remit of ZIKA-VID, it will become clear below that the analysis of persistence cannot be isolated from the analysis of gestational age at maternal infection and the risk of vertical transmission. These analyses must be conducted simultaneously and, as a result, all are covered in the ZIKA-VT SAP (*Sections 6 & 7*). If they are not carried out simultaneously, this would lead to an analysis of test persistence that is not technically consistent with our analysis of VTR.

Further, the ZIKA-VT work on population epidemiology will contribute information about the test sensitivity, specificity and cross-reactivity. An outline of methods for deriving this information from the ZIKA-VT data will be covered in the population epidemiology SAP (*Section 8.2*), but a fuller account will appear in the ZIKA-VID work package.

4. ZIKA-SYN: joint statistical analysis

The statistical analysis plans set out in this document are all specifically designed *to allow for joint analysis alongside other datasets*, both those generated by the other EU-funded ZIKA programs, ZIKA-Plan and ZIKAlliance, and those generated by other non-EU funded consortia such as the ZIP (ZIKA in Pregnancy) study. The joint analysis must of course be absolutely restricted to *bona fide* prospective vertical transmission studies, a definition of which can be found in *Section 5*.

As a necessary first step in the design of an SAP, we now state the *properties* that a statistical analysis must have to meet the requirement of a joint statistical analysis in this context. The SAP must be able to deliver unbiased estimates of the target parameters, regardless of:

1. Differences between studies in the maternal populations studied, for example symptomatic women only, as opposed to all women.
2. Differences between collaborating centers in VTR and risk of adverse clinical outcomes, including residual differences after key covariates are taken into account.
3. Differences within and between centers in the temporal scheduling of laboratory tests to identify maternal infection in pregnancy.
4. Differences between studies or centers, or changes over time, in the tests used to detect maternal infection in pregnancy, including differences in the combinations of tests, or in the type of tests.
5. Difference between studies, or between centers within studies, in test sensitivity, specificity and cross-reactivity.
6. Differences between centers in the availability of surveillance data.

It can be shown that the SAP outlined in the following pages has these properties.

5. Background on vertical transmission rate

Vertical transmission rate can only be estimated from a prospective study. The defining feature of prospective vertical transmission studies is that ascertainment of maternal infection status must be independent of the infection status or clinical outcomes in the fetus, newborn, or child. *This is most easily achieved if the mother's infection status is established before any examination of the fetus or newborn.*

Within that framework, the VTR is estimated by dividing the number of vertically infected children by the total number of women who were infected during pregnancy. However, there are difficulties in defining both the numerator and the denominator, due to the imperfect sensitivity and specificity of the diagnostic tests for ZIKV, CHIKV and DENV, and the imprecisely known gestational age at infection.

For this reason, a ZIKA-VT Diagnostic Expert Group (*Appendix 1*) will be responsible for keeping under review the best available information on: the sensitivity and specificity or all diagnostic tests (PCR, IgM, IgG); and the duration of PCR, IgM, and IgG responses following infection, and for advising on the interpretation of sequences of test results in the mother and offspring. The expert group will be responsible for determining the criteria for maternal infection, the timing of maternal infection, and vertical transmission.

5.1 Denominator for VTR

Women will be classified as infected in pregnancy or not, based on PCR evidence of current infection, IgM evidence of recent infection, IgG evidence of seroconversion or rise in titer, assessed at several time-points during their pregnancy. Those infected in pregnancy form the denominator of the VTR.

To obtain unbiased estimates it may be necessary to take into account that IgM and IgG test are less than 100% sensitive and specific (see *Section 7.6*). There may also be miss-classification of the exposure due to serological cross-reactivity between ZIKV and DENV. Samples that are IgM+ for both are likely to be false positives on one of the two infections.

In order to take information on sensitivity, specificity and cross-reactivity into account in estimating vertical transmission rates and rates of congenital infection without bias, it is necessary to have information on the *a priori* risk of infection. This can be derived from surveillance data and information collected in ZIKA-VT (see *Section 8.1.7*).

5.2 Numerator for VTR

Fetuses will be regarded as vertically infected if fetal or placental tissue, or amniotic fluid, tests PCR+. Children will be regarded as vertically infected on the basis of IgM or PCR evidence within 7 days of delivery. Infants who lose antibody will be regarded as not vertically infected: this includes infants in whom a falling IgG titer is observed as well as those who become IgG-.

There are reports of children with clinical signs of congenital infection, such as microcephaly, whose mothers were infected in pregnancy, *but in whom no laboratory evidence of congenital ZIKV can be obtained from testing serum and CSF for IgM and PCR*. Definitions of congenital zika in the absence of laboratory evidence are being developed [2, 3], which may be revised as new data becomes available. Although the evidence is not clear at the time of writing, it seems likely that these infected children will remain IgG antibody positive.

Infants who are IgG antibody positive when last seen may be congenitally infected. The probability that they are congenitally infected depends on two factors: firstly, their age, because maternal antibody declines over time and is usually absent within 15 months. Secondly, the risk of post-natal

infection. *Appendix 5* describes statistical analyses that use data on antibody loss in control children born to IgG+ mothers, and data on post-natal infection on initially IgG- newborns, to estimate the probability of vertical infection in all IgG+ infants whose infection status cannot otherwise be determined.

It remains possible either that congenital infection can take place, causing damage, and clearing prior to delivery and leaving no laboratory markers; or that maternal infection affects fetal development without crossing the placenta. It is for this reason that ZIKA-VT includes a 2nd control group, of children born to mothers with no infection in pregnancy.

5.3 Gestational age (GA) at maternal infection

In a full data analysis, the target parameter here is not a single date, but a *probability distribution* for each woman, representing when the onset of infection was likely to have occurred, relative to the date of conception which may itself be imperfectly known. To simplify presentation in this SAP we represent this as a discrete distribution in units of one week. Information on the distribution of GA at infection will be derived from one or more of the following

(a) the date of symptoms, if reported

(b) dates of a first PCR+ test result and subsequent PCR results

(c) seroconversion, between last IgG- and first subsequent IgG+

(d) dates of a first IgM+ and subsequent IgM tests

(e) dates of an initial IgG+ followed by a rising IgG titer subsequent IgG tests

(f) avidity tests may also be used to help estimate date of infection, especially in samples that are IgM+ and/or IgG+ at recruitment

Note that an initial PCR+ or IgM+ test result is only informative about timing of infection to the extent that the population distributions of PCR persistence and IgM persistence are known. Thus, test persistence information will be critical in estimating GA at maternal infection (see *Section 7.4*).

In practice, if the tests all had perfect sensitivity and specificity, the sequence of test results would either indicate no maternal infection during pregnancy, or it would define an *interval* during which infection has occurred. The persistence distributions, if relevant, will impact on the probability distribution of GA at infection *within* that interval. The issue of test accuracy adds a further layer of complexity, discussed in the next section.

A third source of information is the weekly local surveillance of incident cases of confirmed or suspected ZIKV, DENV and CHIKV [4]. If we reasonably assume that the attack rates in the general population are proportional to attack rates in the pregnant women being recruited, the surveillance data represents an *a priori* distribution of GA at maternal infection within the interval defined by the sequence of tests. The PCR or IgM persistence distributions, if relevant, will combine with the surveillance information to define maternal GA at infection.

5.4 Taking test sensitivity, specificity and cross-reactivity into account

PCR tests can be regarded as effectively 100% specific, but they have a short detection window, which varies between individuals. We refer to this as the test's persistence distribution. Serological tests vary in their specificity and sensitivity. Failures of specificity (false positives) can arise for several reasons. While some may be due to a random signal that has exceeded the test's threshold, other false positives may be due to cross-reaction to antibodies to related viruses. Currently available DENV and ZIKV IgM tests react to each other's IgM, and to other flavivirus IgM. We can therefore anticipate that ZIKV and DENV IgM test specificity might vary between collaborating centers, reflecting the geographical distribution of other viruses to which they are cross-reactive. In addition, although IgM antibody is usually assumed to persist for 12 weeks, in some individuals it may persist for many months. Therefore, if IgM is due to an infection acquired many months before the pregnancy, we might regard it as an *analytic* true positive, but a *diagnostic* false positive.

Information on the effective sensitivity and specificity of IgG and IgM assays in different centers is currently incomplete, but will be systematically developed under the ZIKA-VID program. Sources of data on properties of diagnostic tests, for use in ZIKA-VT data analyses, will be updated regularly (see *Appendices 3 & 4*).

Reliable estimates of the sensitivity and specificity of each individual test are not, by themselves, enough to determine the probability that a woman has experienced an infection in pregnancy given her test results. Each woman will be tested on multiple occasions, and on multiple tests. Interpretation of these sequences of tests is complex as we can anticipate there are strong correlations, conditional on true infection status, both between tests results at the same time, and between tests of the same type over time. Rules for determining whether a sequence of tests and clinical findings should be considered as a reflecting a "confirmed", "definite" or "possible" maternal infection will be drawn up by the Expert Diagnostics Group (*Appendix 1*).

In order to use information on test sensitivity and specificity, we must also take into account the *absolute* risk of infection in each pregnant woman during the period when she is pregnant. A ZIKV IgM+ result is more likely to be a false positive if there are few surveillance reports of ZIKV at that time; and more likely to be a DENV infection if large numbers of DENV are being reported. Estimates of the *temporal trends* in infection of ZIKV, DENV and CHIKV are available from routine surveillance sources, as noted above; to obtain estimates of the absolute risk surveillance data must be supplemented by data on the proportion of infections, whether symptomatic or not, that are reported to the surveillance center. This proportion, which will vary between ZIKV, DENV and CHIKV, can be *approximately* estimated by aligning weekly surveillance reports with data from ZIKA-VT (See *Section 8.1*).

Following reports of sexual transmission of ZIKV, ZIKA-VT will be collecting information on infection in fathers / partners. We will explore the possibility of using this information alongside surveillance data when estimating the likely timing of maternal infection.

6. Vertical transmission risk and outcomes: basic statistical outputs

Basic analyses, without attempts to correct for sensitivity, specificity and cross-reactivity, and making only the simplest assumptions about test persistence, are set out as a series of table shells. The most likely trimester of maternal infection in these tables would be derived from rules based on dates of tests and assumptions about test persistence.

Table 1 provides crude estimates of the risk of vertical transmission and the risk of congenital anomalies, by *most likely* trimester of presumed maternal infection, and by symptoms.

Table 2 provides a simple listing of congenital anomalies.

Table 3 is a breakdown of outcomes of pregnancy (fetal death, stillbirth) by indicators of maternal infection in pregnancy.

Table 4 represents a generic form of analysis for delivery data and subsequent pediatric outcomes, separate for ZIKV, DENV, and CHIKV, and by trimester of infection, and laid out by birth cohort (Vertically infected, Maternal Infection but no vertical infection, No maternal infection). Covariates can be taken into account by multiple regression.

With so many outcomes to examine, in three distinct diseases, the Table 3 and Table 4 analyses must be considered as hypothesis-forming, not hypothesis-testing.

Tables 1-4 will be computed with standard software, such as SAS, STATA, or R. However, as pointed out in Section 5, any analyses involving the denominator for vertical infection, so especially Table 1 estimates, will be vulnerable to very considerable biases due to inaccuracy in identifying the timing of maternal infection, and indeed, whether there was a maternal infection during pregnancy at all.

The methods set out in the next section are designed to solve these problems, leading to gestational age at infection being represented as *probabilities* that infection occurred in the 1st, 2nd, and 3rd trimester, rather than the usual 0,1 “dummy covariates” used in conventional analyses of gestational age as a risk factor.

7. Vertical transmission risk and outcomes: comprehensive statistical analysis

As discussed in the previous section, an analysis of VTR and the risk of severe outcomes among the vertically infected comprises several inter-related components: test persistence; test sensitivity and specificity; surveillance data; gestational age at maternal infection; as well as vertical transmission and outcomes of vertical transmission. The relationships between these evidence sources and the model parameters to be estimated can be set out schematically as an influence diagram (Figure 1). Using the influence diagram, we can describe the separate components of the statistical analysis. We will throughout assume a Bayesian framework. This is partly because prior information on test

persistence, sensitivity, specificity and cross-reactivity plays an essential role in achieving unbiased estimates, and because there is a need to "update" the prior persistence information using the ZIKA-VT data. In addition, GA at maternal infection must be characterized as a *distribution*, which will be informed by a combination of persistence and surveillance data.

7.1 Vertical transmission rate

Assume that for each woman i we will have estimates of the probability $\pi_i(t)$ that the onset of her infection occurred in week t. We assume that vertical transmission can only occur if infection exists during pregnancy, so that the *onset* is in the interval $-2 \le t \le G_i$ where G_i is the gestational age at delivery. If the probability of vertical transmission given maternal infection at week t is $V(t)$, then the probability of *in utero* transmission in women i is:

$$v_i = \sum_t V(t)\pi_i(t), \quad Y_i \sim Bern(v_i) \tag{7.1}$$

The outcome data $Y_i = 0,1$ (1 indicating Vertical infection (VI), 0 indicating no VI in a child born to women i), is a Bernoulli realization of the parameter v_i. In a Bayesian framework, we can attribute a vague prior to $V(t)$ and use the data obtain posterior distributions for $V(t)$, v_i, and $\pi_i(t)$ given the Bernoulli likelihood.

In practice, a smooth parametric or semi-parametric function would be fitted to $V(t)$. The analysis is readily extended to include additional risk factors $\mathbf{X_i}$ with coefficients $\boldsymbol{\beta}$:

$$logit(v_i) = logit\left(\sum_t V(t)\pi_i(t)\right) + \boldsymbol{\beta}^T \mathbf{X}_i$$

7.2 Risk of severe outcomes of congenital infection

A similar form of analysis will be carried out for severe outcomes of congenital infection. The precise definition remains to be determined, but could include: fetal death, stillbirth, microcephaly, cranial disproportion, or ZIKV-related death within the first two years. The analysis will estimate the risk of severe outcomes conditional on vertical transmission having taken place. This clarifies that there may be two distinct processes: risk factors for congenital infection, and risk factors for severe damage given congenital infection.

The form of analysis for severe clinical outcomes conditional on vertical infection parallels that for vertical transmission rate. Here, c_i is the risk of severe outcomes in a child born to women i, and $C(t)$ is the probability of severe outcomes given maternal infection in week t. Data $Z_i = 0,1$ indicating severe (1) or non-severe (0) outcomes is a Bernoulli realization of c_i, and can be used to update $C(t)$ and $\pi_i(t)$:

$$c_i = \sum_t C(t)\pi_i(t), \quad Z_i \sim Bern(c_i)$$

The analysis can be extended to include risk factors as shown above.

7.3 Severe outcomes with no laboratory evidence of in utero transmission

As noted in Section 5, this may possibly represent a distinct outcome, requiring its own analysis with respect to GA at infection and other risk factors. If so, the analysis would be along the same lines as the analyses above. Alternatively, if, as we suspect, persistent antibody is found in these cases, then no special provision is required.

7.4 Analysis of gestational age at maternal infection

We start with an analysis that assumes perfect test sensitivity and specificity. The gestational age at the onset of maternal infection is $\pi_i(t)$, t in weeks. Although the period of interest is between just before conception and delivery, ie the interval $-2 \le t \le G_i$, $\pi_i(t)$ is defined over a wider interval $-2-D \le t \le G_i + D$, where D is the maximum duration of a test of persistence. This is because a first IgM+ test D weeks after delivery, or an IgM- test $D+2$ weeks before conception, both provide information about the probability of infection during pregnancy. Because there may be a finite probability that the maternal infection occurred before conception, $\sum_{-2 \le t \le G_i} \pi_i(t)$ can take any value between 0 and 1. Some special cases help clarify the concepts:

7.4.1 Onset of infection marked by symptoms. If a woman has symptoms in pregnancy starting at T then $\pi_i(t) = 1, \text{ if } t = T$ and $\pi_i(t) = 0, \text{ if } t \ne T$.

7.4.2 Seroconversion alone. If the interval is defined by seroconversion during pregnancy, ie between a last IgG- at t_1 and a first IgG+ at t_2 , then in the absence of any other information,

$$\pi_i(t) = 1/(t_2 - t_1 + 1), \text{ if } t_1 \le t \le t_2, \text{ else } 0.$$

7.4.3 Surveillance data alone. Alternatively, suppose there are surveillance counts $M(t)$ of suspected or confirmed cases (of ZIKV, DENV, or CHIKV) depending on the disease being studied [4], then the "a priori" distribution of when the infection might have occurred (given that it occurred in pregnancy) can be represented as a Dirichlet distribution:

$$\pi_i(t) = \alpha(t), \quad \alpha(t) \sim Dirichlet\big(M(t)\big), \quad -2 \le t \le G_i$$

7.4.4 PCR+ or IgM+, no surveillance data. If the specified interval is defined by a PCR+ or IgM+ test at time t_2 and the earliest possible date of infection was t_1 (which might be marked by an IgM- result, or $t_1 = t_2 - D$) then, if the cumulative persistence distribution (the probability that persistence is d or less weeks) is $F(d, \theta)$ with parameter(s) θ, then (dropping the θ):

$$\pi_i(t) = \frac{F(t_2 - t)}{\sum_{t_1 \le u \le t_2} F(u - t)}, \text{ if } -2 \le t \le t_2, \text{ else } 0$$

7.4.5 PCR+ or IgM+ with surveillance data. Now we assume the same test results, but surveillance data is available as well:

$$\pi_i(t) = \frac{\alpha(t)F(t_2 - t)}{\sum_{t_1 \le u \le t_2} \alpha(u)F(u - t)}, \text{ if } t_1 \le t \le t_2, \text{ else } 0$$

7.4.6 If the infant is congenitally infected, and we have PCR+ or IgM+ with surveillance data as above, we need only consider scenarios in which the maternal infection occurs during pregnancy. The summation in the denominator is therefore over: $Max(t_1, -2) \le u \le Min(t_2, G_i)$

7.5 Using ZIKA-VT data to update prior information on test persistence

The previous section has shown how, given information on test persistence, the date of a positive PCR or IgM test informs the GA of maternal infection. In this section, we show how to estimate the distribution of test persistence from ZIKA-VT data, or, more usefully, how to update any prior information available.

Simplifying assumptions have been made for purposes of clarity and in order to keep the notation minimal: for example, that the date of onset of symptoms is taken to be the date of the onset of infection, and that tests are positive from that date. Adjustments can, and will, be made to accommodate these factors, but at this stage greater veracity would be at the expense of clarity.

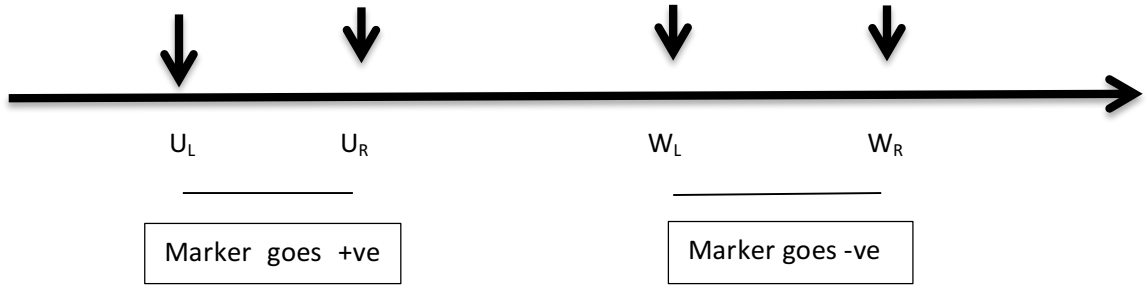
$F(d, \theta)$ is defined for $d = 1, 2 \ldots D$ where D is the maximum possible duration of that marker. The data generated by ZIKA-VT and similar studies is *doubly interval censored*, because in most cases neither the onset of the marker response, nor the end of the marker response, is observed. Methods for analysis of doubly interval censored data have been published [5, 6] and have been applied to the timing of maternal infection in the context of congenital toxoplasmosis [7, 8]. A recent paper [9] includes a brief up-to-date review of the most commonly used methods.

ZIKV and the ZIKA-VT protocol have features that require extensions and modifications to existing methods. First, not only is incidence changing over time, but each individual woman is exposed to a different incidence which varies over the course of her pregnancy. Second, information on multiple markers in multiple types of samples will need to be processed simultaneously.

We begin with an analysis for a single marker, then we consider how to handle variables that might affect persistence, such as previous infection or the presence of symptoms, and finally the joint analysis of multiple markers.

7.5.1 Estimating the persistence distribution of a single test

The objective here is to estimate the parameters θ of the persistence distribution $F(d,\theta)$ from data $U_{Li}, U_{Ri}, W_{Li}, W_{Ri}$ for every woman i, taking the following general form:



This represents a sequence of tests $-++-$, starting with a "last –ve" before the infection occurs at U_L and a first +ve after the infection at U_R. The onset of infection is within this interval. The date at which the test turns negative is between the last +ve at W_L and the first –ve at W_R. In practice, we may not observe a sequence $-++-$ in a series of IgM or PCR tests in every woman. However, in combination with the occurrence of symptoms and the results of IgG tests, it will be possible to construct definite dates $U_{Li}, U_{Ri}, W_{Li}, W_{Ri}$ for every woman i who tests +ve on either IgM or PCR. For example, if symptoms occur at time T then $U_{Li} = U_{Ri} = T$; if no final –ve test is observed, then $W_{Ri} = W_{Li} + D$; if no initial –ve test is observed, then $U_{Li} = U_{Ri} - D$; and so on. Note, also, that if a baby born to women i is confirmed to be congenitally infected, then this information requires that $-2 \le t \le G$; in other words, $U_{Li} \ge -2$ and $U_{Ri} \le G_i$.

If the data indicates that the GA of maternal infection is at t, $U_{Li} \le t \le U_{Ri}$, then the probability of the data given that $t = T$ is:

$$\Pr(U_{L_i}, U_{R_i}, W_{L_i}, W_{R_i} \mid t = T, \theta) = \begin{cases} F(W_{R_i} - T - 1, \theta) - F(W_{L_i} - T - 1, \theta) & U_{L_i} \le T \le U_{R_i} \\ 0 & \text{otherwise} \end{cases}$$

Therefore, averaging over the distribution of maternal infection $\pi_i(t)$, the likelihood for the data is:

$$\Pr(U_{Li}, U_{Ri}, W_{Li}, W_{Ri} \mid \pi_i(t), \theta) = \sum_t \pi_i(t)\big(F(W_{Ri} - t - 1, \theta) - F(W_{Li} - t - 1, \theta)\big),\ U_{Li} \le t \le U_{Ri}$$

If the onset of infection at t_S is observed, $U_{Li} = t_S = U_{Ri}$ and $\pi_i(t_S) = 1$, and the likelihood reduces to the standard likelihood for right censored or right interval censored data.

In a Bayesian framework, we may incorporate already published data on the parameters θ. For example, a persistence distribution has been published for a ZIKV RT-PCR, characterized as a Weibull distribution [10]. Either informative priors for θ based on the published material can be entered, or published results on $F(d,\theta)$ itself and its confidence intervals can be built in, in a form of multiple parameter evidence synthesis [11]; see Guyot [12] for a similar example.

We will check for conflict between the ZIKA-VT data and any prior information by testing the impact of removing the prior information and the use of goodness of fit statistics $\bar{D}$ and DIC [13].

Appendix 3 details the publications which will be used to provide prior information on persistence parameters that will be incorporated in the statistical analysis. This table will be routinely updated.

7.5.2 Form of the distribution of persistence and impact of covariates

Analysis of the persistence distributions usually relies on standard two-parameter distributions, particularly Weibull, Gamma, and Log-logistic, and their 3-parameter generalisations. These parametric curves will be approximated by a piece-wise constant hazard $\mu(d,\theta)$ during each time interval d, which gives us the cumulative distribution function (probability that the duration is $\leq d$) :

$$F(d,\theta) = 1 - \exp\left(- \sum_{u=1\ldots d} \mu(u,\theta) \right)$$

In considering the appropriate form of these distributions, it is important to appreciate that the observed between-individual variation in duration of IgM reflects not only the stochastic uncertainty in how a single survival distribution is realized, but also between-individual variation in survival distributions, due to variation in the inoculum and variation in immune response. To better account for this, semi-parametric functions may also be fitted; for example, a random walk smoothing process applied to $log\mu(d)$

The impact of covariates on persistence will be assessed, including: presence of symptoms, coinfection, and previous infection with the same or other arboviruses. Avidity tests will be used to determine whether an infection is primary or secondary.

A common strategy to study covariates is proportional hazards (PH), which introduces a single additional parameter δ_X for each covariate X to the log hazard. Thus, in the presence of the covariate, $\mu(d,\theta)$ is replaced by $\mu(d,\theta)\, e^{\delta_X}$. Otherwise, if PH is counter-indicated by the data, it can be relaxed and interactions between the covariate and time since infection can be introduced. The alternatives can be evaluated by residual analysis and global goodness of fit.

7.5.3 Estimating the persistence distributions of multiple tests

The ZIKA-VT protocol provides for

(i) PCR, IgM, and IgG tests on women with symptoms,
(ii) PCR on all IgM+, IgG seroconversions, and rises in IgG titer.
(iii) Repeat PCR, IgM, and IgG tests at the prescribed intervals until a first PCR-, IgM-, IgG falling titer, respectively.
(iv) Use of serum, saliva, and urine samples.
(v) Avidity tests may be used to determine if an infection is primary or secondary; this also carries information on timing of infection

Thus, each woman's record is likely to consist of a series of results on at least three tests and possibly more if more than one assay of each type is used.

The analysis above for a single test is readily extended to two or more tests $k = 1, 2 \ldots K$. The data on each individual i now consists of K sets of implied $-++-$ sequences denoted $U_{Li_k}, U_{Ri_k}, W_{Li_k}, W_{Ri_k}$, and the parameter set is expanded to accommodate K distributions $F_k(d, \theta_k)$. The likelihood for the persistence tests for woman i is then:

$$\Pr\left(\underset{k}{} (U_{Li_k}, U_{Ri_k}, W_{Li_k}, W_{Ri_k}) \mid \pi_i(t), \theta_k \right) =$$

$$\sum_t \pi_i(t) \prod_k \left(F_k(W_{Ri_k} - t - 1, \theta_k) - F_k(W_{Li_k} - t - 1, \theta_k) \right) \quad Max_k(U_{Li_k}) \le t \le Min_k(U_{Ri_k})$$

Note that, in this framework, the date of symptoms and the dates of short persistence tests such as PCR, are highly informative about the persistence distributions of longer persistence tests such as IgM and rising IgG.

Extension to multiple tests, use of a variety of tests, and changes in which tests are used, raises questions about the numbers of parameters that may need to be estimated. Our initial strategy will be to assume that the persistence distributions of all tests of the same type (PCR, IgM, rising IgG) have the same underlying parametric or semi-parametric form, with persistence distributions that differ only by a single parameter, for example based on a proportional hazards assumption. This constant would be estimated from the data. Persistence distributions for different *samples* (serum, saliva, urine) on the same test will be treated the same way.

These are strong assumptions, but ones which minimize the number of additional parameters, and which will allow for quite precise estimation of persistence in large datasets. They will be rigorously tested by goodness of fit and residual analyses, and sensitivity analyses will be conducted to establish their impact on results.

7.6 Accounting for imperfect test sensitivity and specificity, and cross-reactivity

As noted earlier, we need to calculate for each woman recruited into ZIKA-VT, the probability that she was infected in pregnancy, and the distribution of GA when this infection occurred, if it occurred at all, based on tests with imperfect accuracy.

For example, a sequence of four ZIKV IgM- results throughout pregnancy, assuming there were no IgG, PCR tests and no symptoms, would be scored as "No evidence of maternal infection in pregnancy" in basic analyses, but there is a possibility that they are all false -ves.

As long as the testing algorithm is relatively sensitive, say 95% or more, the bias in estimates of the vertical transmission due to false -ves is likely to be negligible. A small proportion (<5%) of maternal infections would be missed from the denominator, but their babies will not be followed up, and so would also be missed from the numerator. The exception to this would be if they are not only congenitally infected but their congenital infection is apparent. But it can be shown that, on current evidence, this would lead to only a very slight over-estimation of the VTR.

The difficulties posed by false *positive* IgM tests are, however, far more serious. If the specificity of an IgM test is 95%, then 5% of uninfected mothers will receive false +ve results, and will be considered infected in pregnancy. If 10% of women are infected in pregnancy, then, for every 15 women considered as infected in pregnancy, only (approximately) 10 would be truly infected, and the vertical infection rate will be underestimated by a factor of 1.5. If incidence is lower, and only 1% of women are infected in pregnancy, then only 1 in 5 women being followed are truly infected and the vertical transmission rate will be underestimated by a factor of 5.

The diagnostic algorithm will include confirmatory tests, and it is hoped that the effective specificity and sensitivity of the entire algorithm will be high. However, it may still be necessary to take false positives and negatives into account. Calculations capable of making these adjustments (*see Appendix 2*) assume that an estimate is available of the *absolute a priori* risk of infection, based on surveillance data. This is in contrast with the calculations in Section 7.4 which assumed perfect test accuracy, where only the relative probabilities were needed. The reason for this is that the probability that a ZIKV IgM+ is a false positive will be much higher if there is very little ZIKV circulating at the time, and much greater – depending on the cross-reactivity of the test – if there is a high incidence of DENV. Methods for obtaining information on the absolute incidence are explained in *Section 8.1.7*).

Note that adjustments for sensitivity and specificity need to be based on the properties of the entire algorithm, and on the entire set of results through the pregnancy.

7.7 How the full analysis will be used

The various components discussed above can be assembled into a single analysis, as shown by the influence diagram (*Figure 1*), providing a single integrated analysis of: test persistence and its covariates; GA at maternal infection; VTR and its risk factors; and risk of severe congenital outcomes and its risk factors.

One of the outputs will be the distribution of maternal GA at infection, in weeks. We will extract the posterior mean probability that a maternal infection occurred during each month, or trimester, and take this forward to be used in analyses along the lines of Tables 3 and 4. In effect the 0,1 "dummy covariates" that would be used in conventional analyses of GA at infection as a risk factor, can then be replaced by a set of probabilities. Note that these cannot exceed one, but they need not add to one, if there is a finite probability that the infection occurred before, or after, pregnancy. Standard

statistical software can then be used to explore GA at infection as a risk factor, or interactions between GA at age of infection and other risk factors, exactly as if 0,1 covariates were being used.

7.8 Software

The single integrated analysis can only be carried out in a Bayesian framework. It remains to be decided whether existing Bayesian Markov Chain Monte Carlo packages (WinBUGS, OpenBUGS, JAGS, STAN) will be suitable for this, or other Bayesian computational packages, such as INLA. Given the size of the dataset and the use of non-standard likelihoods, it may be necessary to write a specific Gibbs Sampler and code the problem in C++ to be run on a multiple-processor machine.

8. Population epidemiology of ZIKV, DENV, CHIKV

We set out the SAP for population epidemiology as a series of specifications for tables, providing comments on the definitions of variables and their interpretation.

8.1 Analyses to be carried out

Note that the estimates of incidence and seroprevalence that can be generated directly from ZIKA-VT are not true population estimates, because, within each collaborating center, the intention is to recruit pregnant women from areas at higher risk of arbovirus infection, and during periods of higher incidence. However, there is an interest in estimating incidence because it may correlate with risk of transmission or adverse clinical outcomes, if for example, both are related to viral load or degree of susceptibility.

All analyses will be initially stratified by center and mosquito season, or by center and year if there is no mosquito season. The text under each heading below should be understood as specifying one or more tables, in most cases separately for each of the three arboviruses.

8.1.1 Basic demographics of the sampled population. We will tabulate the numbers recruited each season, their age distribution. Further breakdowns will be by: socio-economic class, income range, type of housing, area of residence, residence stratified by arbovirus infection incidence (from surveillance data), as considered appropriate

8.1.2 Seroprevalence and cumulative incidence. For each arbovirus, the denominator for a seroprevalence estimate is the number of women who had at least one IgG test, and the numerator is the number who were IgG+ *on their first test.* In addition to "crude" (without adjustment) estimates of seroprevalence, we will obtain estimates of cumulative incidence π, which is (observed) seroprevalence p adjusted for IgG sensitivity and specificity, using the relationship

$$p = \pi Se + (1-\pi)(1-Sp)$$

The sensitivity and specificity will be characterized as probability distributions based on the data available, assembled under the ZIKA-VID work package. The sources of this data will be continually updated (see *Appendix 4*).

If more than one test was used, results will be reported separately for each test (see *Table 5*)

The adjustment can be extended to account for cross-reactivity between DENV and ZIKV IgG tests, given information on sensitivity, general false positive rates, and cross-reactivity of both assays:

$$p_Z = \pi_Z Se_Z + (1-\pi_Z)(1-Sp_Z) + \pi_D Xr_Z$$
$$p_D = \pi_D Se_D + (1-\pi_D)(1-Sp_D) + \pi_Z Xr_D$$

This gives us two equations in two unknowns, and allows us to estimate the true cumulative incidence of both infections π_Z, π_D from the observed seroprevalences p_Z, p_D.

To bring together cumulative incidence estimates from multiple tests, the above methods for correcting for sensitivity and specificity can be extended to apply to *any number of tests*, and *any number of combinations of tests (see Appendix 6).*

8.1.3 Proportion of symptomatic infections. For each infection, the denominator is the number of infected women, and numerator is the number of those reporting symptoms (according to pre-specified criteria) who were confirmed by PCR+, IgM+, or rising IgG. Infections which may either be ZIKV or DENV will be tabulated separately. We will investigate whether the proportion of symptomatic infections for each arbovirus is related to seroprevalence of the others.

8.1.4 Seroconversions and incidence of primary infections. The denominator is the number of women who were initially IgG- and who were retested on IgG at a later date. The numerator is the number of these women who were tested IgG+ at some later date. The time at risk is the total time between the first IgG- and either the first IgG+ if there was one, or the time between the first and last IgG- tests. *Incidence of primary infection* is found by dividing the number of seroconversions by total time at risk.

Once again, both crude and sensitivity-specificity-adjusted estimates will be considered, and separate estimates will be derived if there is more than one IgG test, using a similar format to *Table 5.*

Associations between incidence on one arbovirus and previous infections with the same or other arboviruses will be explored

8.1.5 IgM prevalence. The denominator is the number of women who were ever tested by IgM, and the numerator is the number whose first IgM test was a +ve. Separate analyses will be performed for different IgM tests, or tests on different samples.

Sensitivity and specificity will be taken into account, as above, and so will ZIKV-DENV cross-reactivity, to give adjusted estimates of IgM prevalence for each assay. Associations between IgM prevalence on one arbovirus and previous infections with the same or other arboviruses will be explored.

8.1.6 Incidence of primary infections estimated by increase in seroprevalence. If we know or can estimate the cumulative incidence at a starting point T, then cumulative incidence at time t is related to incidence between t and T as follows:

$$\pi(t) = \pi(T) + (1 - \pi(T)\left(1 - \exp\left(-\sum_{T \le u \le t} \lambda(u)\right)\right)$$

This can be modelled, for example assuming piece-wise constant incidence.

For DENV infection, which has been present for much longer, it may be preferable to model age-and time-specific cumulative incidence:

$$\pi(a,t) = \pi(a,T) + (1 - \pi(a,T)\left(1 - \exp\left(-\sum_{T \le u \le t} \lambda(a - T + u, u)\right)\right)$$

Using data from pregnant women, we would model incidence from time T and age 16 upwards.

These calculations assume that cumulative incidence can be estimated by adjusting seroprevalence for sensitivity and specificity as shown in *Section 8.1.2*

8.1.7 Alignment with surveillance data: population estimates of incidence. Once estimates of incidence are available, they can be aligned with surveillance reports to estimate the proportion of incident infections (symptomatic or not) that are reported to the surveillance system. "Alignment" should be interpreted as putting the dates of the tests on the real timescale, so that incidence estimates for ZIKV, DENV, and CHIKV based on ZIKA-VT data can be compared to numbers of cases notified to national or regional surveillance centers, both confirmed and suspected.

If the estimated incidence in some period t is $\lambda(t)$ and $M(t)$ cases are reported in, say, the population of adult women aged 16-44, over the same time period and from the same area where ZIKA-VT is recruiting, then we can calculate the proportion of all infections that were reported over that time period as :

$$\theta(t) = \frac{M(t)}{N(1 - e^{-\lambda(t)})}$$

Where N is the relevant population size. This quantity can be examined over time to see if it remains relatively stable, and can be used in analyses of test persistence and adjustments for sensitivity and specificity needed to estimate the gestational age of maternal infection and vertical transmission rate (*see Sections 5,7*).

Further, once an estimate $\theta(t)$ is available, it can be used to "gross-up" counts $M(t)$, to estimate the true number of infections on a national or regional basis: $M(t)/\theta(t)$.

Sources of surveillance data for each collaborating center are listed in *Appendix 7*. These will be continually updated as new centers are included. The appendix will provide a description of the

protocol under which surveillance for ZIKV, DENV, ZIKV is carried out, including: reporting definitions; criteria for "suspected" ZIKV, DENV, CHIKV; circumstances under which samples are collected and tested; and what tests are used.

8.2 Analyses of properties of diagnostic tests

As noted earlier the ZIKA-VT and ZIKA-VID programs are inter-related and the analyses set out in *Section 7.5* will provide the definitive information on the persistence of IgM and other tests.

However, the population epidemiology data collected by ZIKA-VT also provides an opportunity for *supportive* analyses of test persistence, sensitivity and specificity. Results should be compared with the more formal evaluations undertaken as part of ZIKA-VID, which will comprise evidence from published literature as well as findings from *de novo* field work.

8.2.1 Average persistence of IgM. Given estimates of IgM prevalence (*section 8.1.5*) and incidence of primary infections based on seroconversion (*section 8.1.4*) or changes in cumulative incidence (*section 8.1.6)*, estimates of the average persistence of IgM can be estimated by dividing IgM prevalence by IgM incidence.

We anticipate that IgM persistence may differ in women with previous ZIKV or DENV infections. If these women can be identified by avidity tests it will be possible to generate separate estimates of average IgM persistence for primary and secondary infections

8.2.2 Sensitivity and cross-reactivity of IgM and IgG tests. Estimates of sensitivity will be available for IgM and IgG tests in women with infections confirmed by PCR, and from cases where tests failed to detect infections in mothers of babies in whom congenital infection is demonstrated.

8.2.3 Specificity of IgM and IgG tests. Specificity of IgG tests can only be estimated in populations which are known to have *not* been exposed to the infection in question: this means that data collected in ZIKA-VT is not relevant to estimates of IgG specificity. However, IgM specificity can be studied by observing the relationship between the proportion of tests which are positive and the surveillance data over the previous 12 months. IgM persistence is believed to be about 12 weeks on average, so that an IgM+ occurring at a time when surveillance sources indicate there has been no virus circulating for over 4 or 5 months are highly likely to be false positives from a diagnostic point of view, whether due to persistent antibody, cross-reactions, or other factors; although it will be important to be able to rule out foreign travel before making these inferences. Data of this sort will be of great importance in defining the tails of the persistence distributions of IgM tests

8.3 Computational methods

Basic analyses of population epidemiology outcomes can be carried out in standard software such as SAS or STATA.

Wherever adjustment is required for sensitivity, cross-reactivity or specificity, the analyses are best carried out in Bayesian framework, because adjusted estimates require information on sensitivity and

specificity in the form of probability distributions. Although a frequentist framework can be used, by inverting the relationship in the equations in section 8.1.2, thereby making the true prevalence a function of the observed prevalence, it then becomes technically difficult to prevent the confidence intervals for true prevalence estimates from going outside the (0,1) bounds that probability parameters must fall in.

In addition, combining information from multiple tests (*Appendix 6*) with correct propagation of uncertainty is readily achieved in a Bayesian multi-parameter evidence synthesis framework [11], but would otherwise require special software if frequentist software was to be used. Bayesian analyses will be undertaken in WinBUGS 1.4.3 or OpenBUGS.

9. Clinical outcomes in arbovirus-infected women and the impact of pregnancy

This is mainly a descriptive study of the natural history of ZIKV, DENV and CHIKV acquired in pregnancy. There will also be regular testing and follow-up of a cohort of mothers who did not acquire infection in pregnancy, allowing a three-way comparative element to look at natural history in women acquiring infection during pregnancy and after pregnancy, alongside uninfected controls.

9.1 Analyses to be carried out

9.1.1 Spectrum of initial symptoms and risk factors for symptomatic presentation.

Table 6 represents a sample table shell for analyses of initial symptoms. This can be further examined by regression to see if there are risk factors for the occurrence / non-occurrence of specific symptoms

Table 7 sets out an analysis of risk factors for symptomatic versus asymptomatic presentation. Both univariate and multivariate analyses are envisaged but it is not yet possible to specify which factors should be controlled for in a multivariate setting.

9.1.2 Loss to follow-up.

See Table 8.

9.1.3 Natural history: risk factors for subsequent clinical outcomes.

These analyses focus on the subsequent development of clinical sequelae, following the initial presentation. Because there are time-varying risk factors, including pregnancy and infection status, this is best analyzed as a synthetic case-control study, considering each clinical outcome in turn.

As an illustration, we consider persistent joint pain as an outcome. All cases of persistent join pain will be identified, along with the time since the initial symptom presentation. For each case as many controls will be identified, up to a maximum of 10 (this number may be revised subject to the distribution of risk factors). To qualify as a control, a woman must have been under observation longer

than the matched case without ever having experienced persistent joint pain. Control women will also be matched for center and for age at recruitment to the study, if possible to the nearest one or two years.

Matched logistic regression will then be used identify the role of risk factors in the development of persistent joint pain, including: infection (ZIKV, DENV, CHIKV, or no infection), symptomatic presentation initially, pregnancy, previous infection, socio-economic status or educational status, and so on. Table 9 illustrates the form of the output from this analysis. It is too early to specify which variables should be used to control for in multivariate analyses, and some recoding of variables may be needed.

9.2 Computational methods

Standard software for frequentist methods will be used, such as SAS or STATA.

10. Miscellaneous features of the SAP

10.1 Data checking

Data collection forms will be pseudonymised with the use of a study-specific unique identifier, and data entered into a REDCap (Research Electronic Data Capture) database. Within REDCap, a comprehensive set of data quality checks will be conducted periodically to cross-validate the entire patient record. These will include validation checks (including data type; range), cardinality, consistency checks and logic checks. Data queries and source data verification requests will be generated and relayed back to each participating site, with all changes to data during the cleaning process documented.

10.2 Missing data

With so many variables, and with so many different analyses, it is difficult to make any general comments on methods for handling missing data. The following comments set out our general approach:

1. In the case of diagnostic test variables, the concept of “missing data” is not applicable: for the purposes of determining whether mothers have been infected in pregnancy, and if so when, the methods are designed to deal with any pattern of test results occurring at any time before, during, and after pregnancy, including no tests at all. A probability distribution will be created for every woman reflecting when maternal infection took place and whether it took place. With regards to vertical infection, the analysis will assign every child a probability of being vertically

infected, based on test results, which will be 0,1, or a probability determined by the duration of antibody persistence when last tested.

2. With regards to explanatory variables, all analyses will be based on a "complete data" analysis, which assumes that data is missing without regard to outcomes.

3. Loss to follow-up will be carefully monitored and tabulated in relation to infection status, in both pediatric and maternal follow-up studies.

4. For categorical pediatric outcomes, survival analyses will treat loss to follow-up as censored observations. Alongside these analyses, sensitivity to assumptions about informative censoring will be carried out, including examination of the most extreme assumptions, in which (a) all, or (b) no, censored observations are assumed to reach the endpoint

10.3 Sample size

No formal sample size calculations have been carried out. The relevant size metric for the VT study is the number of women who are infected in pregnancy, rather than the numbers of women to be followed through pregnancy. A general *initial* target number of women infected in pregnancy, to allow reasonable estimates of vertical transmission rate and the risk of severe clinical outcomes, would be approximately 1000.

Currently there is no clear consensus regarding either vertical transmission rates or rates of congenital damage, and differences between centers cannot be ruled out. It would therefore be a further advantage if recruitment could be spread over 6 or more centers, perhaps varying in intensity of ZIKV infection, and/or in DENV incidence and seroprevalence.

Once, say, 600 ZIKV infections in pregnancy have been followed through to ascertainment of vertical infection, sample size should be reviewed, depending on the transmission risk and risk of severe outcomes, and taking into account other analyses that have appeared at that time or are planned. At that point, fresh consideration could be given to the eventual size of the study, and the potential advantages of targeted recruitment of women with specific risk factors.

10.4 Multiplicity

ZIKA-VT is built around two fundamental, pre-specified, and semi-independent outcomes: vertical transmission rate, and the risk of serious adverse clinical outcomes conditional on vertical transmission. Risk factors have also been pre-specified, based on risk factors that are important in other congenital infections

All other outcomes will be considered under the heading of hypothesis formation rather than hypothesis testing. No special measures will be taken for multiple outcomes, although Bonferroni adjustments may be applied to significance tests.

10.5 Interim analyses

Estimates of the key parameters can and should be updated at any time as new data is accumulated, without the need for statistical adjustment. This is an observational study which broadly follows local standard of care, with some additional arbovirus testing in certain groups. Therefore, no "stopping rules" will be applied.

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TABLES

Table 1: Risks of Vertical Transmission and Congenital malformation.

Definitions:

1. Presumed maternal infection based on PCR+, IgM+, Seroconversion or rising IgG titer
2. Trimester, most likely trimester based on test dates and persistence
3. Vertical infection based on IgM+, PCR+ in cord or sample from newborn < 7days old, or persistent antibody.
4. Severe and mild congenital malformation, to be defined

	Trimester of presumed infection	***Symptoms during pregnancy***	***Number of Mothers infected***	***Number Vertically infected***	***Number of Congenital malformations (severe)***	***Number of Congenital malformations (mild)***
ZIKV	*1*	Yes				
		No				
	2	Yes				
		No				
	3	Yes				
		No				
DENV	*1*	Yes				
		No				
	2	Yes				
		No				
	3	Yes				
		No				
CHIKV	*1*	Yes				
		No				
	2	Yes				
		No				
	3	Yes				
		No				

Table 2. Listing of all congenital anomalies at delivery

Including:

Most likely trimester of maternal infection
Ultrasound findings
Outcome of pregnancy: fetal death, stillbirth, livebirth
Gestational age at delivery (GA)
Birthweight, birthweight for GA, head circumference, head circumference for GA.
Description of anomalies at delivery: cranial morphology; brain anomalies; ocular anomalies; congenital contractures; neurological sequelae.

Table 3. Outcomes of pregnancy: generic summary tables and covariate analyses

	Trimester of presumed infection	*Symptoms during pregnancy*	*Number of Mothers in category*	
Vertical infection ZIKV	*1*	Yes		***Outcomes of pregnancy to be tabulated.*** Abnormal ultrasound findings: specific abnormalities, groups of abnormalities, etc. to be tabulated Fetal deaths (1st and 2nd trimester) Fetal deaths (3rd trimester) Livebirths ***Covariates to be considered for regression analysis*** Centre Year - season Co-infections in the mother Previous infection in mother, same or different arbovirus Maternal age Obstetric history (e.g. previous stillbirth, congenital abnormalities) Comorbidities (e.g. sickle cell, diabetes) Smoking /alcohol /drug use SES markers
		No		
	2	Yes		
		No		
	3	Yes		
		No		
Vertical Infection DENV	*1*	Yes		
		No		
	2	Yes		
		No		
	3	Yes		
		No		
Vertical Infection CHIKV	*1*	Yes		
		No		
	2	Yes		
		No		
	3	Yes		
		No		
Maternal infection NO vertical infection ZIKV	*1*	Yes		
		No		
	2	Yes		
		No		
	3	Yes		
		No		
Maternal infection NO vertical infection DENV	*1*	Yes		
		No		
	2	Yes		
		No		
	3	Yes		
		No		
Maternal infection NO vertical infection CHIKV	*1*	Yes		
		No		
	2	Yes		
		No		
	3	Yes		
		No		

Table 4. Outcomes by birth cohort: generic summary tables and covariate analyses

	Trimester of presumed infection	*Symptoms during pregnancy*	*Number of Mothers in category*	*Outcome*	***Outcomes to be tabulated:***
Vertical infection ZIKV	*1*	Yes			Number (%) preterm <37 completed weeks Gestational age (mean, sd) Number (%) livebirths Number (%) neonatal deaths Number (%) deaths at one year Birthweight (mean, sd) Birthweight for GA (mean, sd) Length (mean, sd) Length for GA (mean, sd) Head Circumference (mean, sd) Number (%) SGA Head Circumference for GA (mean, sd) Number (%) severe congenital malformations on delivery Number (%) mild congenital malformations at delivery Number (%) severe clinical outcomes at 1 year Number (%) mild clinical outcomes at 1 year Number (%) auditory problems Number (%) Ophthalmological problems Weight, height, head circumference: at 6m, 12m, 24m (mean, sd) Developmental findings at 6m, 12m, 24m etc. ***Covariates to be considered for regression analysis*** Centre Year - season Co-infections in the mother Previous arbovirus infection in mother, same or different arbovirus.
		No			
	2	Yes			
		No			
	3	Yes			
		No			
Vertical Infection DENV	*1*	Yes			
		No			
	2	Yes			
		No			
	3	Yes			
		No			
Vertical Infection CHIKV	*1*	Yes			
		No			
	2	Yes			
		No			
	3	Yes			
		No			
Maternal infection NO vertical infection ZIKV	*1*	Yes			
		No			
	2	Yes			
		No			
	3	Yes			
		No			
Maternal infection NO vertical infection DENV	*1*	Yes			
		No			
	2	Yes			
		No			
	3	Yes			
		No			
Maternal infection NO vertical infection CHIKV	*1*	Yes			
		No			
	2	Yes			
		No			
	3	Yes			
		No			
No Evidence of maternal infection		Yes			

Table 5. Population epidemiology: estimates of seroprevalence and cumulative incidence by center and year/season, for each IgG assay

Cumulative incidence is seroprevalence adjusted for sensitivity, specificity, and cross-reactivity. The overall estimate will combine all sources of data (see *Appendix 6*).

Centre, Year/season		***ZIKV***	***DENV***	***CHIKV***
IgG test 1	*Number ever IgG tested*	N		
	Number +ve on first test	R		
	Observed seroprevalence	P=R/N (95% Confidence interval)		
	Cumulative incidence (adjusted)			
IgG test 2	*Number ever IgG tested*	N		
	Number +ve on first test	R		
	Observed seroprevalence	P=R/N (95% Confidence interval)		
	Cumulative incidence (adjusted)			
etc.	*Number ever IgG tested*	N		
	Number +ve on first test	R		
	Observed seroprevalence	P=R/N (95% Confidence interval)		
	Cumulative incidence (adjusted)			
Cumulative incidence from all sources combined				

Table 6. Women's follow-up study: spectrum of initial symptoms in pregnant women and not-pregnant controls, by center

Symptoms	***ZIKV***		***DENV***		***CHIKV***		***Covariates in regression analyses***
	Pregnant	*Not pregnant*	*Pregnant*	*Not pregnant*	*Pregnant*	*Not pregnant*	
	N %	N %	N %	N %	N %	N %	
Symptom 1							
Symptom 2							
"							
"							
Symptom combination 1							
Symptom combination 2							
"							
"							
Meets clinical criteria 1							
Meets clinical criteria 2							
"							
"							
No symptoms							

Primary vs secondary infection
Co-infection
Previous infection with same or different arboviruses

Table 7. Risk factors for symptomatic vs asymptomatic presentation

	symptomatic	***asymptomatic***	***Univariate Odds ratios (95% CI)***	***Multivariate odds ratios (95% CI)***
Centre				
1			1.0 (ref)	1.0 (ref)
2				
3				
Maternal age				
<20				
20-24				
25-29				
30+			1.0 (ref)	1.0 (ref)
Pregnant				
Yes				
No			1.0 (ref)	1.0 (ref)
Infection				
ZIKV				
DENV				
CHIKV			1.0 (ref)	1.0 (ref)
Previous infection				
ZIKV				
DENV				
CHIKV			1.0 (ref)	1.0 (ref)
Mother's education				
0-4				
5-9				
10+			1.0 (ref)	1.0 (ref)
etc				

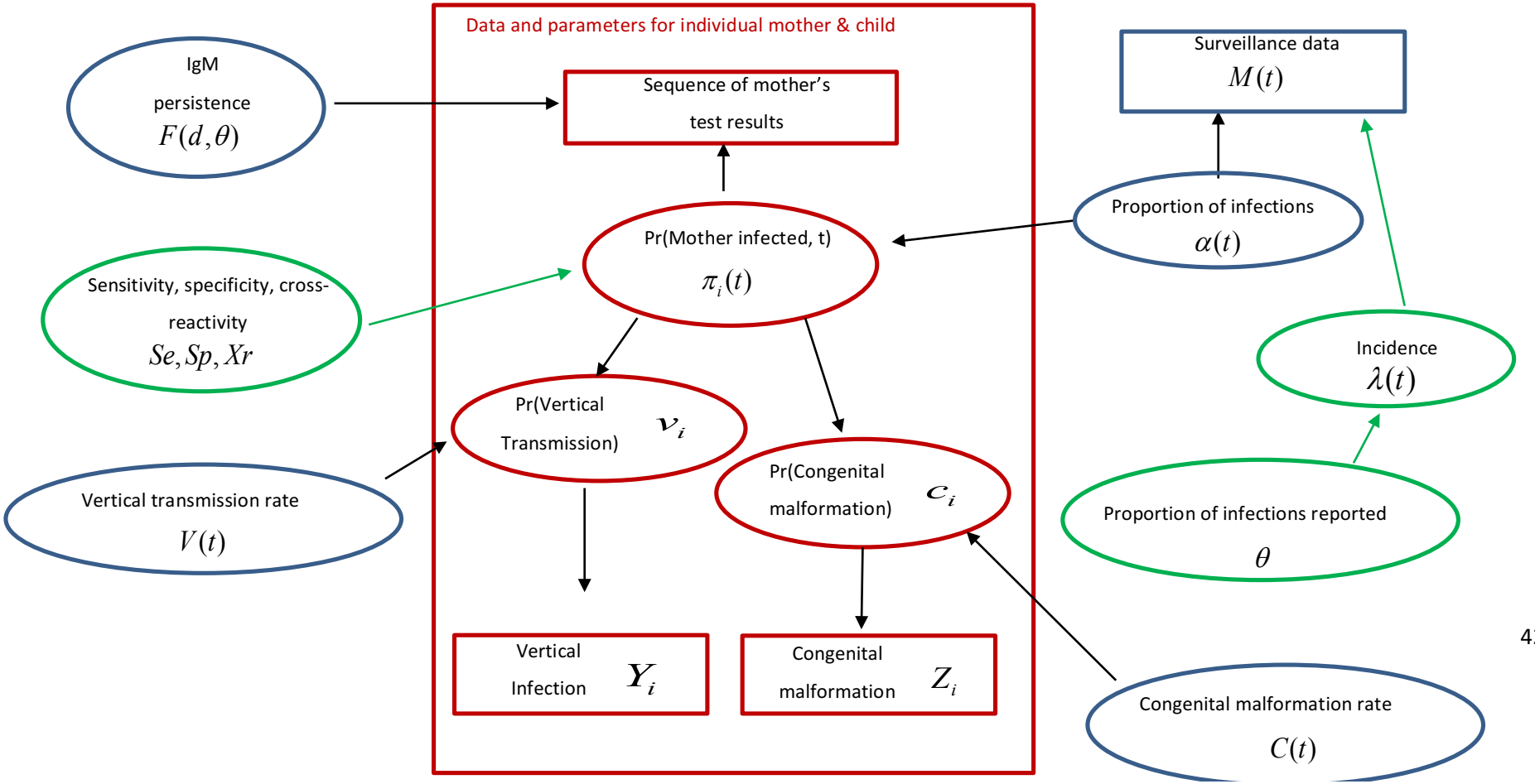
Table 8. Women's cohorts: Completeness of follow up

		Recruitment	***1m***	***4m***	***9m***	***12m***
ZIKV	*Number of women Contributing data*					
	Number lost to Follow-up					
DENV	*Number of women Contributing data*					
	Number lost to Follow-up					
CHIKV	*Number of women Contributing data*					
	Number lost to Follow-up					
Not infected	*Number of women Contributing data*					
	Number lost to Follow-up					

Table 9. Women's follow-up study - Natural history subsequent to initial presentation. Risk factors for a single specified clinical outcome.

Risk factor	***Cases***	***Controls***	***Unadjusted Rel Risk (95% CI)***	***Adjusted Rel Risk (95% CI)***
Infection				
ZIKV				
CHIKV				
DENV				
No infection			1.0 (ref)	1.0 (ref)
Pregnant at symptom onset				
Yes				
No			1.0 (ref)	1.0 (ref)
Pregnant at clinical outcome onset				
Yes				
No			1.0 (ref)	1.0 (ref)
Initial symptoms				
Yes				
No			1.0 (ref)	1.0 (ref)
Mother's Age				
<19				
20-24				
25-29				
30+			1.0 (ref)	1.0 (ref)
Previous infection, prior to the index infection				
ZIKV				
CHIKV				
DENV				
No infection			1.0 (ref)	1.0 (ref)

Figure 1. Schematic influence diagram. Showing the relationships between data and parameters in an analysis of vertical transmission rate and risk of clinical outcomes of vertical transmission. Rectangles denote data and ellipses parameters. Subscript *i* denotes mother-child-specific items, *(t)* denotes dependency on gestational age. Mother-child data and parameters are outlined in red. Items outlined in green and green influence arrows apply only in analyses which include adjustment for sensitivity, specificity and cross-reactivity of persistence tests.



APPENDIX 1. ZIKAction Diagnostics Expert Group

Remit: Within the ZIKA-VT work package, the ZIKAction Diagnostics Expert Group will be responsible for:

1. Deciding with collaborating centers which tests will be used "on-line" in ZIKAction to identify women and newborns for follow-up, and which further tests will be undertaken for the later statistical analyses
2. Keeping under review the performance characteristics of virological and serological tests used in ZIKAction, including, sensitivity, specificity, cross-reactivity; duration of response to current and/or previous infection; keeping under review the impact of previous infection by the same or different arboviruses on these parameters.
3. Formulating algorithms for: the interpretation of sequences of test results – (for example, how to treat a lone IgG –ve in a sequence of IgG+, or vice versa; how to interpret a ZIKV IgM+ DENV IgM+ sample.
4. Reviewing "odd" sequences of test results which lie outside the formulated algorithms, and deciding how these will be treated.

APPENDIX 2. Role of Sensitivity, Specificity, Cross-reactivity in estimating gestational age at infection

We denote sensitivity, specificity, and cross-reactivity as Se, Sp, Xr. Cross-reactivity of, say, ZIKA IgM, is a special kind of false positive rate, in which a positive result on ZIKA IgM is obtained in a person who has not had a recent ZIKV infection but has had a recent DENV infection. For clarity, we distinguish this from other failures of specificity, although they too may be the result of cross-reactions.

The incidence *per week* of ZIKV infection in week t of pregnancy is $\lambda(t)$, so that the probability of an infection during t is $1-e^{-\lambda t}$ and the probability of an infection during a period (t_1, t_2) is

$$1-e^{-\sum_{t_1 \le u \le t_2} \lambda(u)}$$

A2.1 A single ZIKV IgM+ DENV IgM- test

To illustrate, we explore the implications of potentially false positive ZIKV IgM tests which cannot be attributed to DENV. To simplify we assume the DENV IgM is perfectly sensitive so that the -ve result rules out a DENV infection.

We assume a single ZIKV IgM+ DENV- test at week 20, and ask: what is the probability there has been a true maternal infection between weeks -2 and week 20, given a Z+D- IgM result at week 20? This quantity can be written as $p\left(I_{Z,-2:20} \mid Z+D-_{20}\right)$. Let $p\left(\bar{I}_{Z,-2:20}\right)$ be the probability of *no* ZIKV *infection* between weeks -2 and 20. Using Bayes Rule, we expand the formula in *section 7.4.5* to allow for imperfect test accuracy:

$$p\left(I_{Z,-2:20} \mid Z+D-_{20}\right)=\frac{p\left(Z+D-_{20} \mid I_{Z,-2:20}\right).p\left(I_{Z,-2:20}\right)}{p\left(Z+D-_{20} \mid I_{Z,-2:20}\right).p\left(I_{Z,-2:20}\right)+p\left(Z+D-_{20} \mid \bar{I}_{Z,-2:20}\right).p\left(\bar{I}_{Z,-2:20}\right)}$$

$$=\frac{\sum_{-2\le u\le 20} F(22-u).Se\left(1-e^{-\sum_{-2\le u\le 20}\lambda(u)}\right)}{\sum_{-2\le u\le 20} F(22-u).Se\left(1-e^{-\sum_{-2\le u\le 20}\lambda(u)}\right)+(1-Sp).e^{-\sum_{-2\le u\le 20}\lambda(u)}}$$

To calculate $\pi_i(t)$ in this case, we have two components: one for the period up to the IgM+ result, the other after it:

$$\pi_i(t)=\begin{cases}\dfrac{\sum_{-2\le u\le 20} F(22-u).Se\left(1-e^{-\sum_{-2\le u\le 20}\lambda(u)}\right)}{\sum_{-2\le u\le 20} F(22-u).Se\left(1-e^{-\sum_{-2\le u\le 20}\lambda(u)}\right)+(1-Sp).e^{-\sum_{-2\le u\le 20}\lambda(u)}} & -2\le t\le 20\\ 1-e^{-\lambda t} & 21\le t\le G_i\end{cases}$$

A2.2 Extension to allow for cross-reactivity

We now suppose a situation in which a sample is found ZIKV IgM+ *and* DENV IgM+.

In the previous example it was necessary to sum over all the possible times $u,\ -2\le u\le G_i$ when the ZIKV infection might have occurred. To take the possibility of a DENV infection into account, this becomes a two-dimensional problem, in which we must consider all the possible times $u,w;\ -2\le u\le G_i,\ -2\le w\le G_i$ when either infection might have occurred.

$$p\left(I_{Z,-2:20} \mid Z+D+_{20}\right)=$$

$$\frac{p\left(Z+D+_{20} \mid I_{Z,-2:20}\right).p\left(I_{Z,-2:20}\right)}{p\left(Z+D+_{20} \mid I_{Z,-2:20}\right).p\left(I_{Z,-2:20}\right)+p\left(Z+D+_{20} \mid I_{D,-2:20}\right).p\left(I_{D,-2:20}\right)+p\left(Z+D+_{20} \mid \bar{I}_{Z,-2:20}\wedge\bar{I}_{D,-2:20}\right).p\left(\bar{I}_{Z,-2:20}\right).p\left(\bar{I}_{D,-2:20}\right)}$$

$$=\frac{\sum_{-2\le u\le 20} F(22-u).Se\left(1-e^{-\sum_{-2\le u\le 20}\lambda(u)}\right)}{\sum_{-2\le u\le 20} F(22-u).Se\left(1-e^{-\sum_{-2\le u\le 20}\lambda(u)}\right)+\sum_{-2\le w\le 20} F(22-w).Xr\left(1-e^{-\sum_{-2\le w\le 20}\lambda(w)}\right)+(1-Sp).e^{-\sum_{-2\le u\le 20}\lambda(u)}e^{-\sum_{-2\le w\le 20}\lambda(w)}}$$

This formula refers to the sensitivity of the ZIKV IgM and its cross reactivity to DENV IgM, and makes some simplifying approximations regarding the co-occurrence of ZIKV and DENV. The specificity term is the probability of obtaining a Z+D+ result in the absence of a recent infection by either ZIKV or DENV.

A2.3. Extensions to sequences of tests.

In principle, calculations of this sort can be extended straightforwardly to sequences of tests, although they become increasingly tedious. The way in which the sensitivity and specificity of the entire testing algorithm and will be calculated and applied to sequences of tests remains to be determined, so we will not develop the statistical framework further here.

APPENDIX 3. Sources of data on persistence of PCR, IgM tests, IgG rising titer

Study	**ZIKV, DENV, CHIKV**	**PCR**	**IgM**	**IgG rising titer**
Paz-Bailey NEJM 2017 [10]	ZIKV	Triplex RT-PCR		
etc				
etc				

APPENDIX 4. Sources of data Sensitivity, Specificity, Cross-reactivity of diagnostic tests

Study	**PCR, IgM, IgG**	**ZIKV, DENV, CHIKV**	**Sensitivity**	**Specificity**	**Cross-reactivity**

APPENDIX 5. Role of Antibody persistence in diagnosis of congenital infection

The criteria for congenital infection (CI) are: PCR+ in serum, urine or CSF OR IgM+ in serum, urine or CSF; OR congenital zika syndrome meeting an agreed definition (see *Section 5.2*) Infants who lose IgG antibody, or who have consistently falling IgG titers, will be regarded as not CI.

The remaining children are those that remain IgG+ at last follow up. They have a probability of being congenitally infected that increases with time, but it is necessary to take into account that they may have acquired antibody following a post-natal infection.

Suppose we have available information on the probability $Ab(t)$ that an uninfected child born to an antibody +ve mother remains IgG+ t weeks after delivery. Suppose also that we have information on the probability $Pn(t)$ that an initially IgG- newborn is IgG+ t weeks after birth as a result of a postnatal infection.

Now consider the babies born to women infected in pregnancy: all will be initially IgG+. The proportion IgG+ at time t is:

$$X(t) = \big(V + (1-V)Ab(t)\big) + \big(1 - V - (1-V)Ab(t)\big)Pn(t)$$

If there was no post-natal acquisition of antibody, we should observe $X(t)$ fall off to an asymptote V representing the vertical transmission rate. Instead we expect $X(t)$ to fall until all maternal antibody is lost, and then continue to rise in response to postnatal acquisition. As we can observe $Ab(t)$ and $X(t)$, and we have data on and $Pn(t)$, we can recover an estimate of V.

If $AB(t)$ indicates having IgG antibody at time t, then the probability that a child who is IgG+ at age t is CI is:

$$p\big(CI \mid AB(t)\big) = \frac{p\big(AB(t) \mid CI\big).p(CI)}{p\big(AB(t) \mid CI\big).p(CI) + p\big(AB(t) \mid \overline{CI}\big).p(\overline{CI})}$$

$$= \frac{V}{V + \big(1 - Ab(t)\big)V}$$

These analyses can be extended to include covariates, and can be integrated into the calculation of the vertical transmission rate (*Section 7.1*) [14].

Data on $Ab(t)$ will be generated from within ZIKA-VT, but we will also refer to other sources of information as listed in the following table, which will be updated regularly:

Sources of data on loss of maternal antibody

Study	ZIKV, DENV, CHIKV
Ramful, Journal of Infectious Diseases 2013 [15]	CHIKV
etc	
etc	

Appendix 6. Estimating cumulative incidence by adjusting seroprevalence for test accuracy, with multiple tests

We anticipate that seroprevalence estimates will be based on more than one IgG test, and also that the number of tests each woman is tested on may vary. Here we describe how it will be possible to combine data from women tested in the same center/year tested only on IgG assay 1, with data from women tested only on IgG assay 2, *and* data from women tested on both assays, in a single coherent analysis. *The methods can be extended to any number of tests.*

If p_1, p_2 are observed seroprevalence on the two IgG assays, we apply sensitivity and specificity corrections to each, around a common cumulative incidence parameter π, with p_1, p_2 informed by independent Binomial data as discussed previously

$$p_1 = \pi Se_1 + (1-\pi)(1-Sp_1)$$
$$p_2 = \pi Se_2 + (1-\pi)(1-Sp_2)$$

In women who have been tested on both assays, the data is in the form of a cross-classification, which is multinomially distributed: $r_{++}, r_{+-}, r_{-+}, r_{--} \sim Multinomial(p_{++}, p_{+-}, p_{-+}, p_{--}, n)$, where

$$p_{ij} = \pi Se_{ij} + (1-\pi)(1-Sp_{ij}), \quad ij \in (++, +-, -+, --)$$

$Se_{ij}, (1-Sp_{ij})$ are the probabilities of obtaining results ij on the two tests in individuals who have had the infection, and in individuals who have not. This information on sensitivity and specificity will be obtained from studies in the ZIK-VID work package. Where this information is not available, weakly informative prior distributions for sensitivity and specificity of each test conditional on results from other tests, will be constructed based on results obtained where such data is available, and under guidance from the Expert Diagnostics Group.

This analysis can be further extended to account for cross-reactivity between ZIKV and DENV:

$$p_{Zij} = \pi_Z Se_{Zij} + (1-\pi_Z)(1-Sp_{Zij}) + \pi_D Xr_{Zij}$$
$$p_{Dij} = \pi_D Se_{Dij} + (1-\pi_D)(1-Sp_{Dij}) + \pi_Z Xr_{Dij}$$

where Xr_{Zij} is the probability of obtaining a result ij on ZIKV antibody tests IgG-1 and IgG-2 in an individual who has been exposed to DENV (but not ZIKV). This data will be available from ZIKA-VID, or if not, suitably vague priors will be constructed.

Note that, as more tests are added, whether alone or in combination, the number of independent sources of data informing the same target cumulative incidence parameter, increases. This provides an opportunity to check the consistency of estimates based on multiple sources of data, and in effect to check the information on test sensitivity, cross-reactivity, general false positive rate.

Information on test sensitivity should remain constant across centers and seasons: this provides a further powerful check on the reliability of the cumulative incidence estimates.

APPENDIX 7. Sources of Surveillance data

Surveillance Centre	***Data collected***	***Brief protocol including clinical reporting definitions; circumstances under which a sample is collected, circumstances under which a sample is tested, and on which tests.***
Ministry of Health, Jamaica	Confirmed and Suspected ZIKV, DENV, CHIKV, by week of onset of symptoms, age, parish/region.	
Etc.		