

Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment

Diane E Bennett^{1*}, Mark Myatt², Silvia Bertagnolio¹, Donald Sutherland¹ and Charles F Gilks¹

¹World Health Organization, Geneva, Switzerland

²Division of Epidemiology, Institute of Ophthalmology, University College London, London, UK

*Corresponding author: E-mail: bennettd@who.int

Background: The World Health Organization (WHO) HIV drug resistance (HIVDR) threshold survey method was developed for surveillance of transmitted HIVDR in resource-limited countries. The method is being implemented with minimal resources as a routine public health activity to produce comparable results in multiple countries and areas within countries. Transmitted drug resistant HIV strains will be seen first in cities or health districts where antiretroviral treatment (ART) has been widely available for years. WHO recommends countries begin surveillance in these areas.

Methods: Each survey requires ≤ 47 specimens from individuals consecutively diagnosed with HIV to categorize resistance to each relevant drug class as $<5\%$, $5\text{--}15\%$ or $>15\%$. Use of routinely collected information and

remnant specimens is recommended to minimize costs. Site and individual eligibility criteria are designed to minimize inclusion of ARV-experienced individuals and individuals infected before ART was available.

Results: Surveys have been implemented in 21 countries. In this supplement, seven countries report results of $<5\%$ transmitted HIVDR in areas where ART has been available for the longest time period. The main challenges in implementation are acquiring sufficient numbers of eligible specimens and optimizing specimen handling.

Conclusion: The WHO HIVDR threshold survey method is feasible in resource-limited countries and produces information relevant to ART and drug resistance prevention planning.

Introduction

Antiretroviral treatment (ART) programmes are being scaled up rapidly in resource-limited countries [1]. Rapid or uncontrolled emergence of HIV drug resistance (HIVDR) has been widely feared as a potential consequence of ART scale-up [2,3]; widespread transmission of drug-resistant HIV could jeopardize ART expansion efforts.

The extent to which resistant HIV will be transmitted depends on many factors, but one of the most important is ART use, that is, ART coverage in an area, how long it has been used, and the proportion and numbers of ART patients whose regimens are failing [4–6]. In most resource-limited countries, 15–20% of HIV-infected individuals are estimated to be in need of ART [1], but only 28% of those in need were receiving ART as of December 2006; in 2003, the figure was only 2% [1]. Mathematical models suggest that until ART coverage has been widespread for years, $<5\%$ of new HIV infections will consist of transmitted drug-resistant strains and additional conditions will affect the extent to which higher levels are reached

[4–11]. These additional conditions include the percentage of failures with drug-resistant strains, the amount of time patients remain on failing regimens with replicating drug-resistant HIV, the magnitude of viral rebound for patients with drug-resistant strains, the fitness of resistant strains versus wild-type strains and transmission probability per partnership for patients with viral rebound and resistant strains compared with untreated individuals.

Initial fears of widespread ART failure, leading to a high potential for transmission of drug-resistant HIV, have not been borne out. Thus far, even in countries with very limited resources, ART programmes have demonstrated outcomes equal to those seen in clinical cohorts using similar regimens in high-income countries [12–16]. It will be challenging for many countries to maintain optimal outcomes in new ART sites as ART is expanded rapidly to approach universal access. However, because the most ART patients in resource-limited countries are starting on highly potent regimens [17], transmission of drug-resistant

strains on a population basis may be delayed compared with high-income countries, where ART scale-up began with resistance-associated monotherapy and one-class dual therapy [18].

Regardless of regimen potency, some ART programme elements could lead to increased emergence and transmission of resistant strains. In the absence of viral load testing, patients are likely to be maintained on failing regimens with replication of drug-resistant strains for longer periods than in high-income countries [19,20]. Limited availability of second-line regimens [21,22] may also lead to prolonged periods on failing first-line regimens. Quantifying the contributions of these phenomena to transmission of drug-resistant HIV will require additional research. Residual drug activity causing partial viral suppression is generally seen with a regimen to which resistance has developed [23–25], which can lower the relative risk of transmission [26–28] and resistant viruses may be less fit and thus less transmissible [23,29–31]. Behavioural prevention programmes being scaled-up in resource-limited countries may also affect transmission rates. ‘Prevention for positives’ programmes for ART patients have demonstrated reduction in risk behaviours, lowering the risk for HIV transmission [32–36], including transmission of resistant strains.

Regular population-based assessment of transmission of drug-resistant strains is important to assure continued efficacy of the limited ARV regimens in use in resource-limited countries. The World Health Organization (WHO) in collaboration with HIVResNet, a global network of over 50 clinical, laboratory, epidemiology and research experts and organizations, supports an HIVDR prevention and assessment strategy to be implemented by National HIVDR Working Groups in countries scaling up ART [37]. Members of the HIVResNet Epidemiology and Clinical Working Groups have met twice yearly since 2003 to advise the WHO on aspects of strategy development and implementation. This article describes the development of methods for the surveillance of transmitted drug-resistant HIV and issues arising during their initial implementation. A companion article describes the statistical background to the survey methodology [38].

Development of the method

Criteria and rationale

The working groups recommended that the method be developed to meet the criteria specified in Box 1.

Issues considered in development of the methods

It was recognized that compromises would have to be made in the development of survey methods to meet

the criteria. Neither data nor specimens routinely collected in most HIV diagnostic sites in resource-limited countries are ideal for HIVDR surveillance. Specification of eligibility criteria represents a particular challenge because of the paucity of information collected in a standardized format at HIV diagnosis. Another challenge is achieving a sufficiently large sample of relatively recently infected individuals, because few individuals are diagnosed in resource-limited countries until their HIV has progressed to the point of severe disease and ART eligibility [43,44], which is equivalent to 10–12 years post-infection [45–47]. Keeping costs low represents a third challenge, not only because of the high cost of genotyping, but because even minimal changes in routine specimen handling can add substantially to the resources required where infrastructure is limited.

We began the methodology development with the idea of incorporating HIVDR surveillance into HIV Sentinel Serosurveys, which are routinely implemented in resource-limited countries to contribute to HIV prevalence estimates [48]. The surveys take place in specified cities and health planning areas of Asian and African countries using standardized low-cost procedures for data and specimen collection. They are implemented every 1–2 years, most frequently in antenatal clinics (ANC), but also in sexually transmitted infection (STI) clinics and, in countries where HIV prevalence estimates are required for specific subgroups such as injecting drug users (IDU), in sites providing services to specific subgroups. Between 200–1,200 specimens from each area are collected for HIV testing during eligible individuals’ first visit to participating sites during a specified time period. Generally residual specimens collected for another purpose are used: in ANC, these are remnant specimens collected for syphilis testing. A minimum amount of demographic data are collected in a standardized format – age and, for pregnant women, the number of previous pregnancies, are among the data items collected.

The simplicity of the surveys, their focus on specific geographic areas in each country and their incorporation into routine clinical and diagnostic activities made them potentially a useful source for HIVDR surveillance specimens. The age and parity data items support the restriction of HIVDR survey eligibility to individuals <25 years old and to women never previously pregnant. Both criteria potentially limit the number of years of potential exposure to HIV transmission, and the ‘no previous pregnancy’ criterion can also restrict the HIVDR survey sample to women who have not received ARVs for prevention of mother-to-child transmission (PMTCT). However, all resource-limited countries do not perform these surveys and, in countries where HIV prevalence is low, serosurvey specimen

numbers were likely to be insufficient. Our methodology had to be designed to take advantage of HIV sentinel serosurveys where possible, but allow collection of specimens from other sources.

Development of the HIV drug resistance threshold survey concept

With experienced statisticians, we reviewed small area survey techniques used in resource-limited countries to evaluate the prevalence of other health conditions. Binomial sequential sampling, of which the most common example is 'lot quality assurance sampling' (LQAS) [49,50], was selected as likely to meet our needs. LQAS methods are used to evaluate health conditions where prevalences may vary substantially from one geographic area to another within a country and where collection of large samples is problematic. Each survey samples a small number of eligible individuals consecutively encountered in specified sites within an area during a limited time period. Rather than providing a prevalence estimate with confidence intervals, which requires a

larger sample, the surveys support categorization of prevalence as above or below one or more thresholds selected to guide public health actions.

Available sample numbers

The maximum number of eligible specimens specified for the development of the HIVDR surveillance statistical sampling strategy was 50, based on reports from HIV serosurveys and voluntary counselling and testing site (VCT) data from resource-limited African and Asian countries. We found that generally no more than 50 individuals <25 years of age, without previous pregnancies if female, and (where information was available) ineligible for ART, were likely to be diagnosed with HIV in available capital city sites within 3–6 months, even in areas of high HIV prevalence.

Selection of thresholds

Thresholds for transmitted resistance were selected after review of published surveys of transmitted resistance and consultation with HIV clinicians within the

Box 1. Survey method criteria

- **Standard procedures should be specified so that results will be comparable from country to country and over time**
Studies to estimate prevalence of transmitted resistance are common, but a standard methodology is not used that would support comparisons from study to study or calculations of trends. Reviewing limitations of studies in high-income countries, Pillay [8] noted the non-comparability of results, based on differing sampling strategies, prevalence estimates from small surveys without confidence intervals around the estimates, varying laboratory methods and different lists of resistance mutations to define 'resistance'.
 - **The survey methods should be simple and require minimal resources**
Resources are still insufficient in most developing countries to provide HIV care and treatment (including basic laboratory tests) to all who need it and to support HIV prevention. Surveillance costs should be minimized. Elaborate requirements for data collection, specimen handling and testing cannot be supported if surveys are to be performed regularly. In keeping with general principles of public health surveillance [39], surveys should if possible use data and residual material from specimens already being collected during routine clinical or surveillance operations.
 - **Surveys should focus on small geographic areas**
The extent of ART scale-up, and the duration of time ART has been available, differs widely between different cities and health planning areas in most resource-limited countries [1]. Especially while ART scale-up is ongoing, resistance transmission will vary geographically.
 - **The method should maximize the likelihood that participants will have been infected with HIV within the past 3 years and limit the likelihood of previous ARV exposure**
In 2003, <5% of eligible individuals were receiving ART in developing countries [40]. The fact that resistance mutations in transmitted strains persist for many years, used as a justification for surveillance of transmitted resistance in chronically infected individuals in high-income countries [41], is not relevant in areas where ART has been available for very few years. Significant transmission is unlikely to have occurred before ART scale-up.
 - **The required sample size should be small**
The sample size must be minimized to keep costs low and to facilitate surveys in multiple areas of a country, but must be sufficiently large to produce meaningful results.
 - **Specimen collection and handling procedures must be feasible in areas with minimal laboratory resources**
However, procedures must be sufficiently stringent to facilitate amplification and sequencing.
 - **Definition of transmitted resistance should be based on a list of resistance mutations developed and regularly updated as new data become available**
An analysis was done according to principles developed by a working group [17,42]. The group recommended that mutations to evaluate transmitted resistance be associated with treatment with one or more drugs in the drug classes recommended for and known to be used in resource-limited countries where ART scale-up is taking place. The list was also required to be applicable to all HIV-1 subtypes and to minimize the risk of classifying HIV strains with polymorphic mutations as strains with mutations associated with transmitted drug resistance.
-

ART, antiretroviral (ARV) therapy.

WHO HIVResNet Epidemiology and Clinical Working Groups. At the time, a 10% prevalence of transmitted resistance was recommended in high-income countries as the threshold to trigger clinical baseline resistance testing [51,52], based on cost-effectiveness analyses. This recommendation was not considered relevant because ART scale-up in resource-limited countries is based on one first-line regimen selected on a population basis and baseline resistance testing is not feasible. Experts argued that the initial threshold should be lower, to alert public health authorities to take preventive action earlier.

We conservatively selected 5% as the lower threshold level for transmitted drug-resistant HIV. This threshold was based partly on experience in high-income countries: despite nearly 10 years of scale-up with resistance-associated monotherapy or two drugs from one class [53], transmitted resistance was reported at <5% in large surveys in the mid-1990s [54–56]. Blower's models predict that transmitted resistance will not reach 5% in countries where ART is currently being scaled up until after 10 years of scale-up or until >30% of all the HIV-infected population is receiving ART [7,9]. Only 15–20% of HIV-infected individuals are eligible for ART in the majority of resource-limited countries and less than a third of those are currently receiving ART [1], making it unlikely that transmission of resistant strains will reach 5% in the near future if the model is correct.

A second threshold of 15% was selected, based on the agreement of Working Group clinicians that this level represents a point at which the specific mutations being transmitted and their implications for the country's ART regimens should be reviewed.

The method to categorize prevalences was developed based on these specifications and informed by simulations using data from large HIVDR surveys for which precise prevalence estimates and confidence intervals were available. It was validated using data from other surveys. The accompanying article in this supplement describes the development of the statistical approach and details of the analysis method [38].

Survey methods

Sampling

A sample of ≤ 47 eligible individuals consecutively diagnosed with HIV in sites within a survey area is required for the analysis. We advise the collection of 60–70 specimens where possible, to allow for amplification and genotyping problems, and post-diagnosis determinations of ineligibility.

Selection of survey areas

It is recommended that surveys be performed in geographic areas where ART has been available to at

least 20% of eligible individuals for ≥ 3 years. Geographic areas are defined as health planning areas where ART services readily available to residents can be described and differentiated from those available to residents of other areas. The capital city is the most likely area in most resource-limited countries where ART will have been widely available for ≥ 3 years; in many countries, the capital city is the only area where this is the case. If no area in a country meets this criterion, a baseline survey to pilot procedures may be performed in the area where ART has been available for the longest period of time.

Eligibility criteria

Laboratory confirmation of HIV infection, age <25 years old and no previous pregnancies for women are mandatory eligibility criteria in all surveys. If projected sample numbers are sufficient, a more restrictive age criterion of <22 years old is preferred. In areas where sites routinely collect relevant information, or where relevant laboratory tests are available, we recommend that at least one additional criterion be used. The additional criteria were developed to reduce the likelihood of including individuals who were infected for >3 years or who have been previously exposed to ARV drugs, and are listed in Box 2. Identical criteria must be applied in all sites included in one survey within a specific area.

If the National HIV Drug Resistance Working Group in a country believes that a subgroup may potentially have a higher prevalence of transmitted HIVDR than other groups, additional eligibility criteria may be applied to restrict a survey to that group. One example is IDU, because IDU receiving ART are

Box 2. Participant eligibility criteria for HIVDR threshold surveys

Mandatory criteria

- Laboratory confirmation of HIV infection.
- Age <25 years at HIV diagnosis (or age <22 where sample numbers support the more restrictive criterion).
- If female, no previous pregnancy.

Criteria to be applied where information is routinely available

- Documented laboratory evidence of seroconversion.
 - If applicable to the survey group, first risk-defining event within the past 3 years (for example, drug injection or sexually transmitted infections).
 - Documented laboratory evidence of recent infection (if based on a method validated within the country and laboratory context).
 - No previous positive HIV test.
 - No known exposure to antiretroviral drugs.
 - No presumptive or definitive diagnosis of a WHO Stage 3 or Stage 4 clinical event [80].
 - CD4⁺ T-cell count >500 copies/ml [45,81].
-

HIVDR, HIV drug resistance; WHO, World Health Organization.

believed in some countries to be less adherent than other groups [57] and more likely to develop and transmit resistant strains. Because a sample size of 47 is insufficient to categorize resistance transmission in more than one subgroup within one survey, a separate survey should be performed for each subgroup of interest in an area.

Site selection

Guidance on site selection is summarized in Box 3. Site selection should ensure either that attendees over all participating sites are reasonably representative of the area population as a whole or of a subgroup of interest. Every eligible individual attending the site during the period of the survey should be able to be included, which means information on HIV status must be available for all. Where HIV testing is optional and uptake is <95%, a site should not be selected. For instance, in many sites offering PMTCT to pregnant women in resource-limited countries, uptake of HIV testing is poor [58]. Such sites are not suitable because potential participants whose HIV status is unknown cannot be included in the survey; HIVDR prevalence among refusers could differ from that in women accepting tests.

Site selection should further minimize the likelihood that potential participants will have been HIV-infected for more than a few years or that they will be ARV-experienced. Ideal sites are those where records permit identification of individuals who have received

multiple HIV tests and where sufficient numbers of individuals can be identified who have seroconverted within the past 3 years or within a shorter period such as 1 year. Also recommended are sites where a large proportion of individuals testing positive for HIV are likely to be young or recently infected. A large percentage of HIV-positive women attending ANC or PMTCT sites are likely to be <25 years of age [58]. Data from high-income countries suggest that a higher proportion of individuals diagnosed with HIV at STI clinics are recently infected than individuals diagnosed at VCT [59].

Sites where individuals may attend more than once during the period of a survey and where records do not support exclusion of a duplicate specimen on a second visit (for example, anonymous HIV-testing sites or blood donation sites without suitable records) should be excluded. Sites where most individuals seek an HIV test because they are symptomatic are also unsuitable, because most diagnoses will be among persons who were infected before ART was available.

To maintain uniformity of survey procedures, site types should not be mixed within one survey: for instance, sites that exclusively provide antenatal services and sites that exclusively provide STI testing should not be included in the same survey. Sites that offer a variety of services may be included in the same survey, providing each offers the same services. If two different site types are available and if numbers are

Box 3. Site selection criteria for HIV drug resistance threshold surveys

Mandatory criteria

- Based on previously collected information from the available sites in the area, at least 50 (preferably up to 70) specimens from eligible individuals are likely to seek services during a period of 3–6 months.
- Every eligible person attending the sites will have an equal chance of being included in the survey.
 - All individuals receive an HIV test routinely, or
 - Specimens drawn for other purposes are routinely tested as part of an unlinked anonymous HIV serosurvey, or
 - Uptake of optional HIV tests is $\geq 95\%$
- Persons diagnosed with HIV at the sites selected are representative of persons infected with HIV in the area (or a subgroup of interest in that area).
- Records can ensure exclusion of a second HIV-positive specimen from an individual whose first HIV-positive specimen was already collected for the survey.
- A routine blood draw should take place, which can provide a suitable remnant specimen, or an opportunity to draw a minimal amount of blood into a second tube.
- Appropriate procedures to safeguard confidentiality should be in place.
- Unless an unlinked anonymous survey has been approved, site procedures should be able to incorporate informed consent procedures.

Recommended criteria

- Information on previous HIV tests at the sites will be available for individuals tested during the period of the survey.
 - Information on CD4⁺ T-cell count, WHO stage or ART eligibility is routinely recorded for individuals whose HIV tests are positive, or
 - $\geq 20\%$ of individuals whose HIV tests are positive are unlikely to be eligible for ART or have signs and symptoms potentially related to HIV
 - The site is part of a national system that supports identification of previous HIV diagnosis and previous ART at other sites for patients who test HIV-positive during the period of the survey, or
 - Self-reports of previous positive HIV tests and ARV experience are routinely recorded for individuals receiving a HIV test
-

ART, antiretroviral (ARV) therapy; WHO, World Health Organization

sufficient and resources permit, it is advantageous to perform two separate surveys within an area.

Specimen collection and handling

As noted previously, use of remnant material from routinely collected specimens is preferred to minimize costs; their use also increases the likelihood that a specimen will be available for all eligible individuals. Specimens from sentinel HIV serosurveys and HIV specimens from diagnostic sites, such as VCT, are likely to be sera or dried blood spots (DBS). In sites where rapid testing is used and CD4⁺ T-cell counts are drawn immediately after a preliminary diagnosis is made, DBS may be made or plasma may be separated after CD4⁺ T-cell testing is performed. Where remnant specimens are insufficient or unsuitable, it is recommended that an additional tube be drawn during a routine blood draw.

For HIVDR threshold surveys, the minimum recommended specimen volume is 1 ml for plasma or serum, or ≥ 2 DBS (5 preferred) of ≥ 50 μ l (100 μ l preferred). The WHO HIVResNet Laboratory Network Advisory Group recommends that sera or plasma be separated within 48–72 h of the blood draw and frozen at -70°C within 96 h of the blood draw with no subsequent thaws until genotyping. If specimens are transported to another laboratory after freezing, transport should take place on dry ice or liquid nitrogen. Multiple transfers after freezing should be avoided.

DBS should be made from blood drawn into anti-coagulated tubes and should be spotted within 96 h of the blood draw by appropriately trained individuals. DBS may be stored or transported at room temperature for up to 30 days, but preferably no longer than 14 days. Details on specimen handling recommendations can be found in the WHO HIVResNet laboratory article in this supplement [60].

Data collection

Data should be abstracted from information routinely recorded for service provision or sentinel serosurveys. Basic demographic data, data on residence and risk, and relevant clinical and laboratory information are abstracted if available. Age, sex, information on previous pregnancy in women, and date and time of blood draw are the only required data items. Minimal additional information may be collected if its recording will not disrupt service delivery, and if clients agree to provide it.

Ethical issues

Protocols for HIVDR serosurveys must be approved by appropriate national and international ethics committees. In HIV Sentinel Serosurveys, an

unlinked anonymous methodology is generally in place in most countries [61]. Where HIVDR surveillance is to be added to these surveys, WHO recommends requesting ethics committee approval to use the unlinked anonymous process.

In settings where additional information is sought from participants or where additional blood is drawn, informed consent should be required. In some countries, National HIVDR Working Groups and/or ethics committees have decided that consent should be required and results returned even where these conditions do not apply.

Mutation list

A working group developed the list of mutations for evaluation of transmitted HIVDR. The mutations are associated with ART, and are non-polymorphic in all HIV-1 subtypes. The principles are described elsewhere in this supplement [62]. This list has been published [63] and will be next updated in mid-2008.

Quality assurance and analysis

Before the analysis is performed, sequences must undergo evaluation from the WHO Sequence Quality Assessment Tool (http://www.cs.brown.edu/courses/cs190/2005/project_ideas/project_ideas.html), which screens each sequence based on percentages of ambiguous nucleotides, frameshifts, insertions and deletions and stop codons on the nucleic acid level, and for percentages of ambiguous and atypical amino acids and frameshifts on the amino acid level. Sequences with percentages of any of these elements above a specified level are queried. Pairwise genetic distances are also evaluated and sequences that too closely resemble one another are also queried. Laboratories may resolve some problems by re-interpretation, by confirming a close epidemiological relationship between individuals with closely associated sequences or by re-sequencing specimens. Unacceptable sequences are not included in the analysis. An article describing the tool is being drafted for publication.

Chromatograms of sequences with mixtures occurring at resistance-related positions in reverse transcriptase and protease are also re-examined to confirm the presence of a resistance mutation within the mixture.

After sequence queries have been addressed, the sequences are ordered consecutively according to the date and time of blood draw and are evaluated one at a time, using a WHO database tool to identify mutations on the WHO list. The mutations are confirmed using the Stanford website Calibrated Population Resistance application [64], which is based on the same list. The analysis ends as soon as the running total of sequences with one or more relevant mutations is greater than an upper limit or less than a lower limit,

specified by the method for that number of sequences. The derivation of the lower and upper limits for each number of sequences is described in Myatt's article [38]. Categorization of HIVDR prevalence as below or above each of the two thresholds is performed separately for protease inhibitors (PIs), nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs); if prevalence to a class is categorized as >5%, prevalence is categorized for each drug in the class. If no sequences with relevant resistance mutations appear among the first 34 specimens, prevalence can be classified as <5% because the lower limit for 34 specimens is one sequence with a relevant resistance mutation. A categorization of >15% can be based on as few as the first 14 specimens if the number of sequences with relevant resistance mutations among them is higher than the upper limit of five. If prevalence cannot be classified using these minimum numbers of sequences, the analysis continues until a classification can be made. If after 47 sequences are evaluated the number of sequences with relevant resistance mutations is neither below the lower limit (two) or above the upper limit (eight), prevalence is between 5–15%.

If a major resistance mutation is seen in a sequence, eligibility criteria for the individual concerned and for the other individuals included in the survey should be re-examined. If there is no reason to exclude individuals from the analysis, results can be reported.

Reporting and dissemination of results

National HIVDR Working Groups will make annual reports and recommendations to clinicians, other scientists, policy makers and the general public, based on the threshold surveys and other HIVDR assessments. Initial reports from 19 countries are scheduled to appear in 2008. Results should contribute to ART policy decisions, including ART regimen and HIV prophylaxis guidelines. If transmission of resistant strains is seen above either of the thresholds, recommendations should take into account implications of the mutations transmitted for the ART regimens currently used in the country. For instance, evidence of transmitted nelfinavir resistance in a country where the first-line is NNRTI-based and where second-line includes a boosted PI, would have fewer implications than evidence of transmitted NNRTI resistance. Recommendations for appropriate public health actions should be based on survey results seen in the context of information from other HIVDR assessments [37].

Summary of initial survey results

As of March 2008, HIVDR threshold surveys have been completed, or are currently being implemented, in

21 resource-limited countries (Botswana, Cambodia, China, Ethiopia, Ghana, India, Indonesia, Kenya, Malawi, Mozambique, Namibia, Swaziland, Russia, Rwanda, South Africa, Tanzania, Thailand, Uganda, Vietnam, Zambia and Zimbabwe). The summary of results and subsequent discussion will focus on the nine surveys from seven countries reporting results in peer-reviewed articles in this supplement (one survey each from Ethiopia, Malawi, Swaziland, Tanzania and Vietnam; two surveys from South Africa and Thailand respectively) [44,65–70]. Characteristics of these surveys and their results are summarized in Table 1.

Geographic areas and sites selected

Surveys were performed in the capital cities in five of the seven countries and in larger health planning areas in the other two. All areas selected did not meet the requirement for ART coverage of >20% of eligible individuals for ≥ 3 years, but all countries performed surveys where ART was first made available.

In Ethiopia, Swaziland and Tanzania, surveys were performed as an addition to the HIV serosurveys conducted in ANC; in South Africa, stored specimens were evaluated from HIV serosurveys performed previously in 2002 and 2004. In Malawi, PMTCT sites with >95% HIV testing uptake were used. Vietnam and one Thai survey used VCT sites; the other Thai survey was performed at a blood donation clinic where sero-conversion information was available.

Specimens, storage, transport and amplification/genotyping rate

Thailand collected 50 eligible specimens in each of its surveys, Vietnam collected 59 and all other countries collected ≥ 60 . DBS were collected in Malawi and Tanzania, and sera in the remaining countries except in Thailand, where plasma was available.

In South Africa, no attempt had been made in 2002 and 2004, when the stored specimens were collected, to optimize handling methods to facilitate genotyping. Other countries put into place mechanisms to collect and handle specimens according to WHO recommendations, although in Ethiopia, specimens were transported from one facility to another multiple times. Specimens were shipped from Ethiopia, Malawi and Tanzania to genotyping laboratories outside the region, specimens from Swaziland and Vietnam were shipped to respective regional laboratories, and in South Africa and Thailand, genotyping was performed in the country.

South Africa and Ethiopia reported amplification and sequencing rates of 41–52%. In Tanzania and Swaziland, rates were 83–87%. In the remaining five surveys in four countries, amplification and sequencing success ranged from 92 to 94%.

Table 1. Nine HIV drug resistance threshold surveys in seven Countries 2002–2006: characteristics and summary results

Country	Area	Duration	Site type	Consent	Participation (of persons eligible), %	Specimen, number/type	Amplified/ sequenced (%)	Amplification/ sequencing methods	Predominant HIV-1 subtype(s)	Transmitted resistance classification
Ethiopia	Addis Ababa	Apr–Aug 2005	ANC	UA	100%	75/serum	39/75 (52%)/ 39/39 (100%)	Viroseq* or in-house/Viroseq or Trugene [†]	C	<5% (all classes)
Malawi	Lilongwe	Oct–Dec 2006	PMTCT	RC-HIV; UA (HIVDR)	100%	59/DBS	54/59 (92%)/ 34/34 [‡] (100%)	In-house/ In-house	C	<5% (all classes)
South Africa	Gauteng Province	Oct 2002	ANC	UA	100%	128/serum	65/128 (51%)/ 65/65 (100%)	In-house/ In-house	C	<5% (all classes)
South Africa	Gauteng Province	Oct 2004	ANC	UA	100%	117/serum	48/117 (42%)/ 48/48 (100%)	In-house/ In-house	C	<5% (all classes)
Swaziland	Manzini-Mbani Corridor	July–Aug 2006	ANC	UA	100%	70/serum	63/70 (90%)/ 61/63 (97%)	In-house/ In-house	C	<5% (all classes)
Tanzania	Dar Es Salaam	Nov 2005– Feb 2006	ANC	UA	100%	60/DBS	50/60 (83%)/ 39/39 (100%) [‡]	In-house/ In-house	A1, C	<5% (all classes)
Thailand	Bangkok	July 2005– Apr 2006	BD	UA	100%	50/plasma	46/50 (92%)/ 46/46 (100%)	In-house/ In-house	CRF01-AE	<5% (all classes)
Thailand	Bangkok	July 2005– Dec 2005	VCT	UA	100%	50/plasma	46/50 (92%)/ 38/46 (83%)/ 38/50 (76%) [§]	In-house/ In-house	CRF01-AE	<5% (all classes)
Vietnam	Hanoi	Feb–July 2006	VCT	RC-HIV/ IC-HIVDR	99% [¶]	70/serum	49/52 (94%)/ 49/49 (100%)	Trugene	CRF01-AE, CRF15-01B	<5% (all classes)

*Abbott Molecular Genetics, Des Plaines, IL, USA. [†]Bayer Healthcare, Tarrytown, NY, USA. [‡]In these two surveys, additional sequencing was not performed after the number of specimens sequenced was sufficient for the analysis. [§]Amplified and sequenced. [¶]Twelve of 559 individuals were not tested for HIV. Participation rate is based on 71 HIV-positive individuals who met the other criteria. Abbreviations: ANC, antenatal clinics; BD, blood donation site; IC-HIVDR, informed consent for HIV drug resistance survey; PMTCT, antenatal clinics with programme for prevention of mother-to-child transmission; RC-HIV, routine consent for clinical HIV-testing; UA, unlinked anonymous; VCT, voluntary counselling and testing sites.

Prevalence of resistance and resistance mutations

Using the WHO HIVDR threshold survey analysis method, all countries reported <5% prevalence of resistance to PIs, NRTIs and NNRTIs.

Discussion

The WHO-recommended HIVDR threshold survey method was developed to address several needs and constraints specific to developing countries. The method represents a compromise between the need for a standardized, reasonably representative methodology and the necessity to minimize financial and human resources so that surveys can be performed regularly. The reports in this supplement demonstrate that the recommended WHO methodology is feasible though implementation raised several challenges described in the country-specific articles.

Numbers

As expected, achieving sufficient numbers was challenging. All but two countries managed to supply 60–70 eligible specimens, but in Vietnam and Thailand, where HIV prevalence in the general population is <2% [71],

surveys required ≥6 months and numbers were still <60. In African countries, where HIV prevalence is higher, sufficient numbers were achieved in urban areas, but as HIV prevalence decreases [71] and surveys are extended to areas where prevalence is lower [1] numbers may be problematic. Focusing on sites where services are provided to subgroups with higher HIV prevalence in Asia and planning surveys in clinical settings where new ‘provider-initiated’ routine HIV-testing programmes are beginning to diagnose HIV in the early stages of infection, may provide sufficient numbers [35,72].

Amplification and sequencing

Amplification and sequencing rates were >90% from remnant serum, DBS and plasma specimens in three countries and approached that rate (87%) in serum specimens from Swaziland. Their experience suggests that routine specimen handling and laboratory operations can be optimized sufficiently, without instituting high-cost operations. Tanzania identified problems in DBS training associated with the 83% amplification rate that will be addressed in the next survey [70]. South Africa implemented appropriate procedures for

handling of remnant HIV serosurvey specimens for HIVDR surveillance as of 2007 [69]. Investigations are still being carried out to account for the low rate of amplification for Ethiopia – multiple specimen transfers may have allowed unreported specimen thawing. Additional support for specimen handling will be provided in the next survey [65].

Bias

Individuals diagnosed at sites selected for the surveys may not be representative of population infected with HIV in the past 3 years. Still, we believe that by limiting the surveys to specific areas and restricting eligibility we remove several sources of potential bias found in other surveys of transmitted drug resistance [8]. However, individuals infected >3 years before diagnosis, or infected in a different area of the country or by an individual from a different area of the country, may still have been included. WHO is actively supporting improved record-keeping, which fosters many public health benefits and as a by-product will facilitate HIV and HIVDR surveys. New electronic record-keeping systems being initiated at diagnostic sites as well as care sites [43] may make it possible to apply more stringent eligibility criteria and increase representativeness, particularly at provider-initiated HIV-testing sites [73].

Failure to amplify or genotype one or more specimens is a potential source of bias only if characteristics potentially associated with drug resistance are associated with the failure. Specimen handling problems, the most likely source of failure, are unlikely to be related to participant characteristics. Other common sources of failure to amplify or sequence are also unlikely to be associated with drug resistance. Although many resistant strains that emerge in response to inadequate ART may be less fit, those strains that are in fact transmitted are unlikely to be associated with a sufficiently low viral load to impede amplification [74]. Moreover, most surveyed individuals are unlikely to be acutely infected, which may increase the likelihood that drug-resistant strains may already have lost mutations impeding replication such as M184V [75].

Non-inclusion of eligible participants is another potential source of bias. Eight of the nine surveys were unlinked anonymous surveys in which no consent was requested, and specimens were available from all eligible participants. In Vietnam, where consent for HIV testing is required at VCT sites, 12 (2%) of 559 potentially eligible individuals refused HIV testing, and thus could not be assessed for survey eligibility. An additional 12 were HIVDR tested, but did not consent to inclusion in the HIVDR survey, only one of these was HIV positive. Nguyen *et al.* [67]

correctly points out that the lack of results for the non-consenting HIV-positive individual could potentially contribute to bias.

One advantage of our methodology is that this source of bias is quantified and taken into account when evaluating the results. Many high-income country surveys that take advantage of referrals from interested clinicians or stored specimens do not report refusal rates or non-inclusion rates based on the numbers of individuals attending the relevant sites from whom specimens were not included [41,76].

The utility of the ‘no previous pregnancies’ criterion to eliminate one source of bias was inadvertently confirmed in the 2004 South African survey. Sequences from four women with previous pregnancies, diagnosed in sites where NNRTI-based PMTCT had been previously available, were accidentally included in the initial analysis and would have led to an overestimate of transmitted NNRTI-resistance if their eligibility had not been re-evaluated and their results excluded [69].

Context of surveys to evaluate transmission of drug-resistant HIV

Our methodology differs substantially from the European surveillance practice of evaluating transmitted resistance among chronically-infected patients starting ART [41]. We believe it is more likely that baseline resistance in reportedly drug-naïve patients starting ART in resource-limited countries is due to undisclosed ARV experience than to transmitted resistance [20,25].

Although they provide important information, surveys of transmitted resistance are insufficient to guide public health action to minimize transmission of drug-resistant strains. WHO recommends that in addition to surveys to evaluate transmission, countries should assess both baseline resistance and previous ARV experience before the start of first-line ART at sentinel ART sites [77]. Systematic evaluation of HIVDR ‘early warning indicators’ to assess ART programme functioning in all treatment sites [37,78] and cohort surveys to monitor the emergence of drug-resistant strains during ART at sentinel sites [77] are also recommended. Results from this combination of assessments should support evidence-based recommendations to strengthen appropriate prescribing, drug supply continuity, adherence and HIV transmission prevention, as well as to guide ART policy.

Future plans for surveillance of transmitted drug-resistant HIV

WHO and its HIVResNet partners are committed to supporting and providing technical assistance for HIVDR transmission surveillance resource-limited countries. Funding is provided by the WHO, the Bill and Melinda Gates Foundation, and numerous donor

countries, academic institutions and other non-governmental organizations.

In 2008, HIVResNet working groups will re-evaluate aspects of the survey methodology. The statistical modelling subgroup will assess whether the thresholds of <5%, 5–15% and >15% are appropriate based on new information, and work with the clinical and epidemiology groups to recommend actions based on prevalence categorization. Studies and mathematical modelling will also be performed to evaluate the extent and duration of ART availability that should trigger an initial survey, methods to evaluate the need for specific surveys for subgroups and methods to select representative sentinel geographic areas, so that surveys need not be performed in all areas of a country after ART coverage is countrywide.

Additional validation of the threshold survey method by more extensive surveys is a high priority. Validation surveys in resource-restricted countries are currently likely only to confirm that the method is valid where there is little or no transmitted resistance, so evaluations are also taking place in areas of known high HIVDR transmission in high-income countries. [79] Low-cost larger representative survey methods, for countries where these are indicated and feasible, are also being explored.

Conclusion

Initial evidence from diverse resource-limited countries using the WHO HIVDR threshold survey method suggests that in the first years of ART scale-up, transmission of drug-resistant strains of HIV is likely to be limited and that the method can be implemented in areas with minimal infrastructure. As ART is expanded within each country, HIVDR transmission surveys using standardized methods should be expanded to additional areas and performed routinely. Data provided by these surveys, in conjunction with other assessments, will provide important information to limit the emergence and transmission of resistance in resource-limited countries.

Acknowledgements

International Medical Press is acknowledged for their support with publishing this manuscript. Some of the authors are staff members of the World Health Organization. The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions or stated policy of the World Health Organization

Disclosure statement

The authors declare that they have no competing interests.

References

1. World Health Organization, Joint United Nations Program on HIV/AIDS, and UNICEF. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector. Progress Report, April 2007 (accessed 5 January 2008). Available from http://www.who.int/hiv/mediacentre/universal_access_progress_report_en.pdf.
2. Popp D, Fisher JD. First, do no harm: a call for emphasizing adherence and HIV prevention interventions in active antiretroviral therapy programs in the developing world. *AIDS* 2002; **16**:676–678.
3. Wadman M. Experts clash over impact of cheap AIDS drugs in Africa. *Nature* 2001; **410**:615–616.
4. Baggaley RF, Garnett GP, Ferguson NM. Modelling the impact of antiretroviral use in resource-poor settings. *PLOS Med* 2006; **3**:e124.
5. Blower S, Brodine E, Kahn J, McFarland W. The antiretroviral rollout and drug-resistant HIV in Africa: insights from empirical data and theoretical models. *AIDS* 2005; **19**:1–14.
6. Vardavas R, Blower S. The emergence of HIV transmitted resistance in Botswana: “when will the WHO detection threshold be exceeded?”. *PLoS ONE* 2007; **2**:e152.
7. Vardavas R, Blower S. Antiretrovirals, Africa and the evolution of drug-resistant HIV: predictions for Botswana. *Antivir Ther* 2005; **10**:S154.
8. Pillay D. Current patterns in the epidemiology of primary HIV drug resistance in North America and Europe. *Antivir Ther* 2004; **9**:695–702.
9. Blower S, Ma L, Farmer P, Koenig S. Predicting the impact of antiretrovirals in resource-poor settings: preventing HIV infections whilst controlling drug resistance. *Curr Drug Targets Infect Disord* 2003; **3**:345–353.
10. Blower SM, Aschenbach AN, Gershengorn HB, Kahn JO. Predicting the unpredictable: transmission of drug-resistant HIV. *Nat Med* 2001; **7**:1016–1020.
11. Blower SM, Aschenbach AN, Kahn JO. Predicting the transmission of drug-resistant HIV: comparing theory with data. *Lancet Infect Dis* 2003; **3**:10–11.
12. Ivers LC, Kendrick D, Doucette K. Efficacy of antiretroviral therapy programs in resource-poor settings: a meta-analysis of the published literature. *Clin Infect Dis* 2005; **41**:217–224.
13. Severe P, Leger P, Charles M *et al.* Antiretroviral therapy in a thousand patients with AIDS in Haiti. *N Engl J Med* 2006; **353**:2325–2331.
14. Ferradini L, Jeannin A, Pinoges L *et al.* Scaling up of highly active antiretroviral therapy in a rural district of Malawi: an effectiveness assessment. *Lancet* 2006; **367**:1335–1342.
15. Wools-Kaloustian K, Kimaiyo S, Diero L *et al.* Viability and effectiveness of large-scale HIV treatment initiatives in sub-Saharan Africa: experience from western Kenya. *AIDS* 2006; **20**:41–48.
16. Sow PS, Otieno LF, Bissagnene E *et al.* Implementation of an antiretroviral access program for HIV-1-infected individuals in resource-limited settings: clinical results from 4 African countries. *J Acquir Immune Defic Syndr* 2007; **44**:262–267.
17. Gilks CF, Crowley S, Ekpini R *et al.* The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. *Lancet* 2006; **368**:505–510.
18. Fischl MA, Richman DD, Griego MH, *et al.* The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. *N Engl J Med* 1987; **317**:185–191.
19. Cozzi-Lepri A, Phillips AN, Ruiz L *et al.* Evolution of drug resistance in HIV-infected patients remaining on a virologically failing combination antiretroviral therapy regimen. *AIDS* 2007; **21**:721–732.

20. Pillay D, Kityo C, Robertson V *et al.* Emergence and evolution of drug resistance in the absence of viral load monitoring during 48 weeks of Combivir/Tenofovir within the DART Trial. *4th IAS Conference on HIV Pathogenesis, Treatment, and Prevention*. 22–25 July 2007, Sydney, Australia. Poster 642.
21. Ford N, Wilson D, Costa CG, Lotrowska M, Kijtiwatchakul K. Sustaining access to antiretroviral therapy in the less-developed world: lessons from Brazil and Thailand. *AIDS* 2007; **21 Suppl 4**:S21–S29.
22. Brief Summary of CHAI Second-Line Pricing Analysis, August 2007 (accessed 10 October 2007). Available from <http://www.clintonfoundation.org/pdf/chai-arv-price-analysis-082407.pdf>.
23. Deeks SG. Treatment of antiretroviral-drug-resistant HIV-1 infection. *Lancet* 2003; **362**:2002–2011.
24. Eron JJ Jr, Bartlett JA, Santana JL *et al.* Persistent antiretroviral activity of nucleoside analogues after prolonged zidovudine and lamivudine therapy as demonstrated by rapid loss of activity after discontinuation. *J Acquir Immune Defic Syndr* 2004; **37**:1581–1583.
25. DART Virology Group and Trial Team. Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa. *AIDS* 2006; **20**:1391–1399.
26. McCormick AW, Walensky RP, Lipsitch M *et al.* The effect of antiretroviral therapy on secondary transmission of HIV among men who have sex with men. *Clin Infect Dis* 2007; **44**:1115–1122.
27. Quinn TC, Brookmeyer R, Kline R *et al.* Feasibility of pooling sera for HIV-1 viral RNA to diagnose acute primary HIV-1 infection and estimate HIV incidence. *AIDS* 2000; **14**:2751–2757.
28. Gray RH, Wawer MJ, Brookmeyer R *et al.* Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet* 2001; **357**:1149–1153.
29. Fellay J, Boubaker K, Ledergerber B *et al.* Prevalence of adverse events associated with potent antiretroviral treatment: Swiss HIV cohort study. *Lancet* 2001; **358**:1322–1327.
30. Back NK, Nijhuis M, Keulen W, *et al.* Reduced replication of 3TC-resistant HIV-1 variants in primary cells due to a processivity defect of the reverse transcriptase enzyme. *EMBO J* 1996; **15**:4040–4049.
31. Clavel F, Race E, Mammano F. HIV drug resistance and viral fitness. *Adv Clin Pharmacol* 2000; **49**:41–66.
32. Bunnell R, Ekwaru JP, Solberg P, *et al.* Changes in sexual behavior and risk of HIV transmission after antiretroviral therapy and prevention interventions in rural Uganda. *AIDS* 2006; **20**:85–92.
33. Bunnell R, Mermin J, DeCock KM. HIV prevention for a threatened continent: implementing positive prevention in Africa. *JAMA* 2006; **296**:855–858.
34. Holstad MM, DiIorio C, Magowe MK. Motivating HIV positive women to adhere to antiretroviral therapy and risk reduction behavior: the KHARMA Project. *Online J Issues Nurs* 2006; **11**:5.
35. World Health Organization and Joint United Nations Program on HIV/AIDS. Guidance on provider-initiated HIV testing and counseling in health facilities. May 2007 (accessed 5 January 2008). Available from http://whqlibdoc.who.int/publications/2007/9789241595568_eng.pdf.
36. Kennedy C, O'Reilly K, Medley A, Sweat M. The impact of HIV treatment on risk behaviour in developing countries: a systematic review. *AIDS Care* 2007; **19**:707–720.
37. Bennett DE, Bertagnolio S, Sutherland D, Gilks CF. The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. *Antivir Ther* 2008; **13 Suppl 2**:1–13.
38. Myatt M, Bennett DE. A novel sequential sampling technique for the surveillance of transmitted HIV drug resistance by cross-sectional survey for use in low resource settings. *Antivir Ther* 2008; **13 Suppl 2**:37–48.
39. Centers for Disease Control and Prevention. Updated guidelines for evaluating public health surveillance systems. *MMWR* 2001; **50 (RR13)**:1–35.
40. World Health Organization. Progress on global access to HIV antiretroviral therapy: a report on '3 by 5' and beyond, March 2006 (accessed 5 January 2008). Available from http://www.who.int/hiv/progreport2006_en.pdf.
41. Wensing AM, van de Vijver DA, Angarano G, *et al.* Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. *J Infect Dis* 2005; **192**:958–966.
42. Beck EJ, Vitoria M, Mandalia S, Crowley S, Gilks CF, Souteyrand Y. National adult antiretroviral therapy guidelines in resource-limited countries: concordance with 2003 WHO guidelines? *AIDS* 2006; **20**:1497–1502.
43. Stringer JSA, Zulu I, Levy J *et al.* Rapid scale-up of antiretroviral therapy at primary care sites in Zambia. *JAMA* 2006; **296**:782–793.
44. Somi GR, Kibuka T, Diallo K, *et al.* Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania. *Antivir Ther* 2008; **13 Suppl 2**:77–82.
45. Glynn JR, Sonnenberg P, Nelson G, Besterd A, Shearer S, Murray J. Survival from HIV-1 seroconversion in Southern Africa: a retrospective cohort study in nearly 2000 gold-miners over 10 years of follow-up. *AIDS* 2007; **21**:625–632.
46. Collaborative group on AIDS incubation and HIV survival including the CASCADE EU Concerted Action. Time from HIV-1 seroconversion to AIDS and death before widespread use of highly-active antiretroviral therapy: a collaborative re-analysis. *Lancet* 2007; **355**:1131–1137.
47. Morgan D, Okongo JM, Lubega R, Whitworth JA. HIV-1 infection in rural Africa: is there a difference in median time to AIDS and survival compared with that in industrialized countries? *AIDS* 2002; **16**:597–603.
48. UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance. Guidelines for conducting HIV sentinel serosurveys among pregnant women and other groups. December 2003 (accessed 10 October 2007). Available from http://data.unaids.org/Publications/IRC-pub06/jc954-anc-serosurveys_guidelines_en.pdf?preview=true.
49. Lanata CE, Black RE. Lot quality assurance sampling techniques in health surveys in developing countries: advantages and current constraints. *World Health Stat Q* 1991; **44**:133–139.
50. Lemeshow S, Taber S. Lot quality assurance sampling: single- and double-sampling plans. *World Health Stat Q* 1991; **44**:115–132.
51. Holzer C, Youle M. Economic implications of HIV-1 resistance testing in overall clinical care. In *Combination Therapy of AIDS*. Edited by E De Clercq & A-M Vandamme. Basel, Switzerland: Birkhauser Verlag AG 2004; pp.195–204.
52. Weinstein MC, Goldie SJ, Losina E *et al.* Use of genotypic resistance testing to guide HIV therapy: clinical impact and cost-effectiveness. *Ann Intern Med* 2001; **134**:440–450.
53. Saravolatz LD, Winslow DL, Collins G *et al.* Zidovudine alone or in combination with didanosine or zalcitabine in HIV-infected patients with the acquired immunodeficiency syndrome or fewer than 200 CD4 cells per cubic millimeter. Investigators for the Terry Bein Community Programs for Clinical Research on AIDS. *N Engl J Med* 1996; **335**:1099–1106.
54. Weinstock H, Respass R, Sweeney S *et al.* Prevalence of mutations associated with antiretroviral drug resistance among HIV-1 seroconverters in the United States, 1992–1997. *Abstr Intersci Conf Antimicrob Agents Chemother Intersci Conf Antimicrob Agents Chemother* 1998; **38**:422.
55. Weinstock H, Respass R, Heneine W *et al.* Prevalence of mutations associated with reduced antiretroviral drug susceptibility among human immunodeficiency virus type 1 seroconverters in the United States, 1993–1998. *J Infect Dis* 2000; **182**:330–333.

56. Little SJ, Daar ES, D'Aquila RT *et al.* Reduced antiretroviral drug susceptibility among patients with primary HIV infection. *JAMA* 1999; 282:1142–1149.
57. Lucas GM, Griswold M, Gebo KA, Keruly J, Chaisson RE, Moore RD. Illicit drug use and HIV-1 disease progression: a longitudinal study in the era of highly active antiretroviral therapy. *Am J Epidemiol* 2006; 163:412–420.
58. Hladik W, Masupu K, Roels T *et al.* Prevention of mother-to-child transmission and voluntary counseling and testing programme data: what is their utility for HIV surveillance? *AIDS* 2006; 19 Suppl 2:S19–S24.
59. Schwarcz S, Weinstock H, Louie B *et al.* Characteristics of persons with recently acquired HIV infection. *J Acquir Immune Defic Syndr* 2007; 44:112–115.
60. Bertagnolio S, Derdelinckx I, Parker M, *et al.* World Health Organization/HIVResNet Drug Resistance Laboratory Strategy. *Antivir Ther* 2008; 13 Suppl 2:49–57.
61. Diaz T, De Cock K, Brown T, Ghys PD, Boerma JT. New strategies for HIV surveillance in resource-constrained settings: an overview. *AIDS* 2005; 19 Suppl 2:S1–S8.
62. Shafer RW, Rhee S-Y, Bennett DE. Consensus drug resistance mutations for epidemiological surveillance: basic principles and potential controversies. *Antivir Ther* 2008; 13 Suppl 2:59–68.
63. Shafer RW, Rhee S-Y, Pillay D *et al.* HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. *AIDS* 2007; 21:215–221.
64. Stanford University HIV Drug Resistance Website Team. Calibrated population resistance tool (Updated 15 September 2007. Accessed 8 January 2008). Available from <http://cpr.stanford.edu/cpr/>.
65. Abegaz WE, Grossman Z, Wolday D *et al.* Threshold survey evaluating transmitted HIV drug resistance among public antenatal clinic clients in Addis Ababa, Ethiopia. *Antivir Ther* 2008; 13 Suppl 2:89–94.
66. Kamoto K, Aberle-Grasse J on behalf of Members of the Malawi HIV Drug Resistance Task Force. Surveillance of transmitted HIV drug resistance with the World Health Organization threshold survey method in Lilongwe, Malawi. *Antivir Ther* 2008; 13 Suppl 2:83–87.
67. Nguyen HT, Duc NB, Shrivastava R, *et al.* HIV drug resistance threshold survey using specimens from voluntary counselling and testing sites in Hanoi, Vietnam. *Antivir Ther* 2008; 13 Suppl 2:115–121.
68. Sirivichayakul S, Phanuphak P, Pankam T, O-Charoen R, Sutherland D, Ruxrungtham K. HIV drug resistance transmission threshold survey in Bangkok, Thailand. *Antivir Ther* 2008; 13 Suppl 2:109–113.
69. Pillay V, Ledwaba J, Hunt G *et al.* Antiretroviral drug resistance surveillance among drug-naïve HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004. *Antivir Ther* 2008; 13 Suppl 2:101–107.
70. Maphalala G, Okello V, Mndzebele S *et al.* Surveillance of transmitted HIV drug resistance in the Manzini–Mbabane corridor, Swaziland, in 2006. *Antivir Ther* 2008; 13 Suppl 2:95–100.
71. Joint United Nations Programme on HIV/AIDS and World Health Organization. AIDS Epidemic Update December 2007 (accessed 5 January 2008). Available from http://data.unaids.org/pub/EPISlides/2007/2007_epiupdate_en.pdf.
72. DeCock KM, Bunnell R, Mermin J, *et al.* Unfinished business-expanding HIV testing in developing countries. *N Engl J Med* 2006; 354:440–442.
73. Steen TW, Sipone K, Hox Gomez F *et al.* Two and a half years of routine HIV testing in Botswana. *J Acquir Immune Defic Syndr* 2007; 44:484–488.
74. Leigh Brown AJ, Frost SD, Mathews WC *et al.* Transmission fitness of drug-resistant human immunodeficiency virus and the prevalence of resistance in the antiretroviral-treated population. *J Infect Dis* 2003; 187:683–686.
75. Brenner BG, Routy JP, Petrella M *et al.* Persistence and fitness of multidrug-resistant human immunodeficiency virus type 1 acquired in primary infection. *J Virol* 2002; 76:1753–1761.
76. Little SJ, Holte S, Routy JP *et al.* Antiretroviral-drug resistance among patients recently infected with HIV. *N Engl J Med* 2002; 347:385–394.
77. Jordan MR, Bennett DE, Bertagnolio S, Gilks CF, Sutherland D. World Health Organization surveys to monitor HIV drug resistance prevention and associated factors in sentinel antiretroviral treatment sites. *Antivir Ther* 2008; 13 Suppl 2:15–23.
78. Hedt B, Wadonda-Kabondo N, Makombe S *et al.* Early warning indicators for HIV drug resistance in Malawi. *Antivir Ther* 2008; 13 Suppl 2:69–75.
79. Bennett DE, McCormick LM, Prachand N *et al.* Categorization of transmitted HIV drug resistance using the WHO/CDC HIV drug resistance threshold survey method. *Antivir Ther* 2006; 11:S116.
80. World Health Organization. WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children 2007 (accessed 5 January 2008). Available from <http://www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf>.
81. Mellors JW, Munoz A, Giorgi JV *et al.* Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997; 126:946–954.