Recommendations for the follow-up of study participants with breakthrough HIV infections during HIV/AIDS biomedical prevention studies

Paige Etter[a], Raphael Landovitz[b], Sengeziwe Sibeko[c], Magdalena E. Sobieszczyk[d], Sharon A. Riddler[e], Carissa Karg[f], Athe Tsibris[g] and Jeffrey Schouten[a]

Objective: To facilitate collection of cumulative data on longitudinal HIV disease outcomes during HIV prevention studies by developing recommendations for follow-up of the relatively few study participants with breakthrough infections.

Design: We formed a working group to compare and contrast the various approaches taken in recent HIV prevention trials, to summarize the advantages and disadvantages associated with each, and to explore the feasibility of developing protocols for the long-term follow-up of seroconverters.

Methods: We reviewed study designs, objectives, and assessments in 15 interventional studies that followed HIV seroconverters. Protocol team members joined discussions of the various approaches and developed recommendations.

Results: Most HIV prevention clinical trials share a core set of objectives, including the description/comparison of virological, immunological, and clinical course of HIV, and sometimes a comparison of preseroconversion and postseroconversion behavior. Long-term follow-up of seroconverters can be conducted in separate studies if the transition from parent protocol is effectively managed.

Conclusion: We recommend the development of harmonized seroconverter protocols. Although specific research questions in the postseroconversion period may differ depending on prevention modality, harmonizing key evaluations would create an opportunity to ask overarching questions that inform the prevention field with respect to design and implementation of future combination prevention studies. Follow-up immediately postseroconversion should be conducted in the parent protocol before roll over into a follow-up protocol. Development of specimen repositories with ample volumes for future assays, standardized definitions of infection, diagnosis and seroconversion dates, and harmonization of study objectives and sample collections at key time points are important.

AIDS 2013, **27**:1119–1128

Keywords: clinical trials as topic, HIV, HIV seroconversion, pathogenesis, prevention

[a]Office of HIV/AIDS Network Coordination, Fred Hutchinson Cancer Research Center, Seattle, Washington, [b]AIDS Clinical Trials Group, University of California, Los Angeles, California, USA, [c]Centre for the AIDS Programme of Research in South Africa, NR Mandela School of Medicine, University of KwaZulu Natal, Congella, South Africa, [d]HIV Vaccine Trials Network, Department of Medicine, Division of Infectious Diseases, Columbia University College of Physicians and Surgeons, New York, New York, [e]Microbicides Trials Network, University of Pittsburgh, Pittsburgh, Pennsylvania, [f]HIV Vaccine Trials Network Core Operations, Fred Hutchinson Cancer Research Center, and [g]AIDS Clinical Trials Group, Harvard Medical School, Boston, Massachusetts, USA.

Correspondence to Jeffrey Schouten, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, E2-112, Seattle, WA, USA.

Tel: +1 207 667 5980; fax: +1 206 667 6366; e-mail: jschoute@fhcrc.org

Received: 13 October 2012; revised: 6 December 2012; accepted: 10 December 2012.

DOI:10.1097/QAD.0b013e32835dc08e

Introduction

The natural history of HIV infection has been an area of intense study since the start of the epidemic. Initially, and throughout the epidemic, HIV-1 seroconverters were followed longitudinally to describe the clinical, virological, and immunological trajectory of treated [1,2] and untreated HIV disease [3–6] and transmission [7]. Early studies also focused on psychosocial and behavioral factors associated with HIV transmission and infection [8,9]. Long-term follow-up of HIV-infected individuals in natural history studies sheds light on issues such as host genetic factors of resistance and long-term nonprogression [10,11], the impact of treatment on disease progression [12], HIV-1 transmission during and after seroconversion [13], and the effects of a positive test on sexual risk behavior [14]. Some groups followed seroconverters within the context of breakthrough infection studies conducted in high-risk populations not exposed to biomedical preventions to meet a variety of study objectives [15–18], and pathogenesis work during acute infection and long-term follow-up has elucidated the concept of 'founder viruses' in the establishment of HIV infection [19,20].

As it became clear that $CD4^+$ T-cell counts [21,22] and plasma HIV RNA [22,23] during the first 6 months of infection were strong predictors of disease progression [24], the need for expensive long-term follow-up of seroconverters in HIV-1 prevention clinical trials was reduced for a time. For example, the HIV Prevention Trials Network's HPTN 052, which showed that antiretroviral therapy (ART) drastically reduces the sexual transmission of HIV in heterosexual serodiscordant couples [25], did not follow seroconverters after obtaining initial samples and assessments. Also, behavioral prevention studies have not typically needed long-term follow-up of seroconverters to obtain study endpoints, which focused on the primary endpoint – incident HIV infection [26,27].

More recently, however, vaccine and preexposure prophylaxis (PrEP) studies have sought to understand the effects of biomedical interventions on correlates of disease progression [28,29]. In addition, as HIV-infected individuals age, describing development of HIV-associated non-AIDS-related conditions in these cohorts of seroconverters who received PrEP and/or a vaccine may become important. Elucidating the correlations of HIV-associated inflammation will also require longitudinal data from seroconverters from diagnosis through ART. Increasingly, answering these contemporary research questions requires the short- and long-term follow-up of study participants who become infected with HIV-1[30], especially in phase 3 trials of partially effective biomedical prevention modalities.

Some networks, such as the HIV Vaccine Trials Network (HVTN) [31,32] and the Microbicide Trials Network (MTN) [33], have already developed studies for the long-term study of participants who seroconverted while in follow-up during late phase prevention trials. However, harmonization of study protocols that follow seroconverters in such a way that would allow for cross-study analysis remains a challenge. Depending on the nature of the study question, standardizing follow-up, and collection of data at key postinfection time points may yield increased statistical power, but more importantly it would create an opportunity to ask overarching questions about the course of HIV infection in individuals who participated in prevention trials, such as development of drug resistance, change in risk behavior, secondary transmission, and development of non-AIDS-related complications. The ability to ask these questions in a systematic fashion will inform design of future intervention studies and demonstration projects. We therefore conducted a comparative analysis of recent or current HIV biomedical prevention studies to answer the questions: How can retention of participants who acquire HIV in a prevention study and enroll in a seroconverter study be maximized; What study objectives can and should be answered by the short-term and long-term follow-up of seroconverters; and, Which biological specimens and assessments can and should be recommended for all studies that follow seroconverters.

Methods

The leaders of the National Institutes of Health (NIH)-funded HIV/AIDS clinical trials networks requested that the Office of HIV/AIDS Network Coordination (HANC) convene senior representatives of study teams of network prevention studies that follow seroconverters [31–38] to compare and contrast consent and retention approaches, objectives, evaluations, and sample collections, and to prepare recommendations. The Seroconverter Study Group (SSG), comprised eight members, held a total of nine teleconference discussions during which the protocols were reviewed and recommendations were developed. Non-network prevention studies were also considered and senior representatives of these protocols were invited to join group discussions on an ad hoc basis [39–43].

Results

Study design and operational details

A total of 15 studies that follow seroconverters were reviewed and compared. Table 1 provides an overview of these studies and Table 2 compares the aspects of study design that pertain to seroconversion. The studies represented a wide variety of prevention modalities, including microbicides, oral PrEP, and vaccines in phase 1

Table 1. Description of studies: geographic setting, study size and population, status, and duration of follow-up.

Study type	Study	Participant age	Participant population	Location(s)	Estimated study size	Study start date	Study status as of 2 July 2012	Duration of follow-up for nonseroconverters (months)
Microbicide studies	CAPRISA 004	18–40	WSM	ZA	889	May 2007	Concluded	≤30; avg. 18
	TRAPS	**18–40**	**WSM**	**ZA**	**98**	**November 2007**	**Ongoing**	**n/a**
	MTN-003	18–45	WSM	Sub-Saharan Africa	5000	August 2009	Closed to accrual	14–38
	MTN-015	**Varies**	**WSM**	**MW, UG, ZA, ZM, ZW**	**500**	**August 2008**	**Enrolling**	**n/a**
Oral PrEP studies	FEM-PrEP	18–35	WSM	KE, TZ, ZA, MW, ZM	3900	May 2009	Closed to Follow-Up	14
	FEM-PrEP Sxr Substudy	**18–35**	**WSM**	**KE, TZ, ZA, MW, ZM**	**55**	**May 2009**	**Closed to follow-up**	**n/a**
	HPTN 067	≥18	WSM, MSM	TH, ZA	360	August 2011	Enrolling	8.5
	HPTN 069	≥18	MSM	US	400	Pending	Pending	11.5
	iPrEx	Varies	MSM	PE, EC, US, BR, TH, ZA	2499	July 2007	Closed to accrual	11–33
	iPrEx OLE	Varies	MSM	PE, EC, US, BR, TH, ZA	1500	June 2011	Closed to enrollment	19
	Partners PrEP	18–65	HIV neg. partners in discordant couples	KE, UG	3900	October 2009	Closed to follow-up	25–37
Vaccine studies	**HVTN 403**	**Varies**	**W, M**	**BR, PE, US, ZA**	**54**	**April 2002**	**Closed to follow-up**	**n/a**
	HVTN 404	**Varies**	**W, M**	**HT, PE, US, ZA**	**80**	**July 2008**	**Enrolling**	**n/a**
	HVTN 505	18–50	MSM[a]	US	2200	May 2009	Enrolling	60
	HVTN 802	**Varies**	**W, M**	**DO, HT, PE, US, ZA**	**230**	**July 2008**	**Enrolling**	**n/a**

Seroconverter rollover studies are bolded. BR, Brazil; CAPRISA, Centre for the AIDS Programme of Research in South Africa; DO, Dominican Republic; EC, Ecuador; FEM-PrEP, Preexposure Prophylaxis Trial for HIV Prevention among African Women; HT, Haiti; HVTN, HIV Vaccine Trials Network; iPrEx, Iniciativa Profilaxis Preexposicion (Prexposure Prophylaxis Initiative); KE, Kenya; M, men; MTN, Microbicide Trials Network; MW, Malawi; n/a, not applicable; OLE, open-label extension; PE, Peru; PrEP, preexposure prophylaxis; TRAPS, Tenofovir gel Research for Advancing Prevention Science; TZ, Tanzania; UG, Uganda; US, United States; W, women; WSM, women who have sex with men; ZA, South Africa; ZM, Zambia; ZW, Zimbabwe.
[a]Ad5 nAb negative, circumcised, also includes M → F transgender.

to 3 testing. Duration of follow-up postseroconversion differs from study to study. In some cases, seroconverters are followed for a limited time in the 'parent' study (e.g., MTN-003) before or while being transferred to a separate 'rollover' study that only follows seroconverters (e.g., MTN-015); in other cases, the seroconverters are followed entirely within the parent study [e.g. Iniciativa Profilaxis Preexposicion (Prexposure Prophylaxis Initiative) iPrEx] (see Table 2).

Seroconverter rollover studies typically enroll seroconverters from the parent study as soon as possible after HIV diagnosis. However, the timing is usually flexible to allow for maximum enrollment. The duration of follow-up also varies. Although perhaps for different reasons, both vaccine and biomedical studies follow seroconverters after ART initiation.

The method used to determine HIV diagnosis, infection, or seroconversion (Table 2), and frequency of HIV testing (Table 3) were compared across studies. Most studies made use of a diagnosis date, although Preexposure Prophylaxis Trial for HIV Prevention among African Women (FEM-PrEP) estimated infection date and Centre for the AIDS Programme of Research in South Africa (CAPRISA 004) estimated seroconversion date.

Objectives

We compared the objectives for each study (Table 4). Some interpretation and generalization was necessary to develop a meaningful comparison. The most common objectives, which were to describe/compare the virological, immunological, and clinical course of disease and to assess drug resistance, are shown in Table 4 along with three other objectives pertaining to behavioral outcomes, evaluation of response to ART and development of specimen repositories, which were less common but of particular interest to the SSG. There were also a variety of virological, immunological, mutation/drug resistance, and other objectives that were used in only one or two studies; these additional objectives typically related to the intervention type (see Supplemental Digital Content 1 and 2, http://links.lww.com/QAD/A297, detailed objectives of each protocol).

Assessments and samples

Assessments and samples collected were reviewed for each study (Table 3). Whole blood, serum/plasma, and peripheral blood mononuclear cells (PBMC), at least one type of genital or rectal secretion or biopsy, and urine samples were commonly collected. Although few protocols included study objectives specific to behavior, most studies conducted ongoing behavioral assessments. Only three studies included study objectives for development of specimen repositories, but all studies collected at least serum/plasma for storage.

The frequency and volume of blood draws varied across protocols, but the trends were similar. Clinic visits and blood draws are frequent in the weeks and months following HIV diagnosis and become less frequent as time passes. The most intense period of follow-up is typically the first 3 months. See Supplemental Digital Content 3, http://links.lww.com/QAD/A297 for more detailed information about the frequency of HIV testing and visits.

Table 2. Description of studies: seroconverter follow-up.

Study type	Study	Sxr rollover study	Parent study	Associated parent study(ies)	Definitions of infxn, dx, or sxn dates, as used in the study	Transition point from parent study	Number of new infxns during study	Duration of follow-up postsxn (months)
Microbicide studies	CAPRISA 004	−	+	n/a	Sxn: Midpoint date between last negative and first positive antibody test OR 14 days prior to first positive confirmatory PCR, whichever is earlier	n/a	98	3
	TRAPS	**+**	**−**	**CAPRISA 004**	**n/a**	**HIV dx**	**n/a**	**Until ART**
	MTN-003	−	+	n/a	Dx: Specimen collection date of first positive antibody test	n/a	217	14–38, or until MTN 015
	MTN-015	**+**	**−**	**MTN trials (e.g., MTN 003, 018, 020); HPTN 035**	**n/a**	**ASAP**	**n/a**	**Indefinite**
Oral PrEP studies	FEM-PrEP	−	+	n/a	Infxn: Midpoint between date of last negative sample and first positive sample by PCR	n/a	68	≤12
	FEM-PrEP Sxr Substudy	**+**	**−**	**FEM-PrEP**	**n/a**	**HIV dx**	**n/a**	**12**
	HPTN 067	−	+	n/a	Dx: Date of first positive RNA or antibody test	n/a	ukn	≤12
	HPTN 069	−	+	n/a	Dx: Date of first positive RNA or antibody test	n/a	n/a	≤11.5
	iPrEx	−	+	n/a	Dx: Date of first positive antibody test	n/a	100	11–33
	iPrEx OLE	−	+	n/a	Dx: Date of first positive antibody test	n/a	ukn	19
	Partners	−	+	n/a	Dx: Date of first positive antibody test	n/a	78	12
Vaccine studies	**HVTN 403**	**+**	**−**	**Phase 1 & 2 vaccine trials**	**n/a**	**ASAP**	**n/a**	**60–79**
	HVTN 404	**+**	**−**	**Phase 1 & 2a vaccine trials**	**n/a**	**ASAP**	**n/a**	**≤ 84; Until ART or CD4 <200**
	HVTN 505	−	+	n/a	Dx: Date of visit with first detectable viral load but negative antibody test	n/a	ukn	17
	HVTN 802	**+**	**−**	**HVTN 503, 504, 505**	**n/a**	**ASAP**	**n/a**	**≤ 144**

Seroconverter rollover studies are bolded. +, yes; −, no; ASAP, as soon as possible after HIV diagnosis; CAPRISA, Centre for the AIDS Programme of Research in South Africa; dx, diagnosis; FEM-PrEP, Preexposure Prophylaxis Trial for HIV Prevention among African Women; HVTN, HIV Vaccine Trials Network; iPrEx, Iniciativa Profilaxis Preexposicion (Prexposure Prophylaxis Initiative); infxn, infection; MTN, Microbicide Trials Network; n/a, not applicable; infxn infection; OLE, open-label extension; PrEP, preexposure prophylaxis; sxn, seroconversion; TRAPS, Tenofovir gel Research for Advancing Prevention Science; ukn, unknown.

Table 3. Sample collections, assessments and visit frequency in protocols that follow seroconverters.

	Microbicide studies				Oral PrEP studies							Vaccine studies			
Study type Protocol	CAPRISA 004	**TRAPS**	MTN-003	**MTN-015**	FEM-PrEP	**FEM-PrEP Sxr substudy**	HPTN 067	HPTN 069	iPrEx	iPrEx OLE	Partners	**HVTN 403**	**HVTN 404**	HVTN 505	**HVTN 802**
Frequency of HIV tests until dx (weeks)	4	**n/a**	4	**n/a**	4	**n/a**	2–4	2–8	4	4–12	4	**n/a**	**n/a**	4–12	**n/a**
Frequency of visits after dx or screening/enrollment (weeks)	1–12	**1–12**	4	**2–26**	1–4	**4**	4–12	2–8	4–12	12	12	**4–26**	**26**	2–4	**2–26**
Max blood volume after dx (ml)	165	**165**	28	**86**	20	**30**	20	20	53	63	21	**250**	**135**	155	**140**
Genomics information collected? Separate consent needed?	−	**+/+**	−	**−**	−	**−**	−	−	+/+	−	+/−	**−**	**−**	+/−	**+/+**
Whole blood	+	**+**	+	**+**	+	**+**	+	+	+	+	+s	**+**	**+**	+	**+**
Serum/plasma	+s	**+s**	+s	**+s**	+s	**+s**	+s	+s	+s	+s	+s	**+s**	**+s**	+s	**+s**
PBMC	+s	**+s**	−	**+s**	+	**+**	+s	+	+s	+s	−	**+s**	**+s**	+	**+s**
Genital/rectal secretion and/or biopsy	+s	**+s**	+s	**+s**	+	**+**	−	+	+sub	+sub	+s	**+**	**−**	+	**−**
Urine	+	**+**	+	**+**	+	**+**	+s	+	+	+	+s	**−**	**+**	−	**+**
Dried blood spots	−	**−**	−	**−**	−	**−**	+s	−	−	+s	−	**−**	**−**	−	**−**
Hair	−	**−**	+sub	**−**	−	**−**	+s	+s	+sub	+sub	−	**−**	**−**	−	**−**
Oropharyngeal swab	−	**−**	−	**−**	−	**−**	−	−	+sub	+sub	−	**−**	**−**	−	**−**
Ongoing behavioral risk assessment	+	**+**	+	**+**	+	**+**	+	+	+	−	+	**−**	**−**	+	**+**

Seroconverter rollover protocols are bolded. dx, diagnosis; n/a, not applicable; +, yes; −, no; CAPRISA, Centre for the AIDS Programme of Research in South Africa; dx, diagnosis; FEM-PrEP, Preexposure Prophylaxis Trial for HIV Prevention among African Women; HVTN, HIV Vaccine Trials Network; iPrEx, Iniciativa Profilaxis Preexposicion (Prexposure Prophylaxis Initiative); infxn, infection; MTN, Microbicide Trials Network; OLE, open-label extension; PBMC, peripheral blood mononuclear cells; PrEP, preexposure prophylaxis; s, stored; sub, substudy.

Table 4. Incorporation of recommended objectives.

	Microbicide studies				Oral PrEP studies							Vaccine studies			
Study type Study	CAPRISA 004	**TRAPS**	MTN-003	**MTN-015**	FEM-PrEP	**FEM-PrEP Sxr substudy**	HPTN 067	HPTN 069	iPrEx	iPrEx OLE	Partners	**HVTN 403**	**HVTN 404**	HVTN 505	**HVTN 802**
Describe/compare virological course of disease	+	**+**	−	**+**	+	**+**	+	−	+	+	+	**+**	**+**	+	**+**
Describe/compare immunological course of disease	+	**+**	−	**+**	+	**+**	−	−	+	+	+	**+**	**+**	+	**+**
Describe/compare clinical course of disease	+	**+**	−	**+**	−	**+**	−	−	+	−	+	**+**	**+**	+	**+**
Assess drug resistance	+	**+**	+	**+**	+	**+**	+	+	+	+	+	**−**	**−**	−	**−**
Describe/compare the psychosocial and behavioral changes	−	**−**	−	**+**	+	**−**	−	−	+	−	−	**−**	**−**	−	**−**
Evaluate virological and/or immunological response to ART	−	**−**[a]	−	**+**	−	**−**	−	−	−	−	−	**+**	**−**	−	**+**
Provide a repository of specimens that can be used for future analyses	−	**+**	−	**+**	−	**−**	−	−	−	−	−	**+**	**+**	−	**−**

Seroconverter rollover studies are bolded. +, yes; −, no; ART, antiretroviral therapy; CAPRISA, Centre for the AIDS Programme of Research in South Africa; FEM-PrEP, Preexposure Prophylaxis Trial for HIV Prevention among African Women; HVTN, HIV Vaccine Trials Network; iPrEx, Iniciativa Profilaxis Preexposicion (Prexposure Prophylaxis Initiative); infxn, infection; MTN, Microbicide Trials Network; OLE, open-label extension; PrEP, preexposure prophylaxis; sub, substudy.
[a]Participants initiating ART are enrolled into CAPRISA 009.

Discussion

Various organizations conducting HIV prevention trials have been committed to following individuals who seroconvert while enrolled in HIV prevention studies by either incorporating 3–38 months of follow-up into the parent study or offering enrollment in a separate seroconverter rollover study. Such studies have enabled evaluation of clinical, immunological, virological, drug resistance, and other outcomes. A common protocol design and/or common protocol, such as a network-specific or cross-network seroconverter study for the long-term follow-up of study participants who seroconvert while participating in a prevention study, can ease aggregation of data. A common protocol design would need to consider consent, retention, study objectives, sample collections and assessments, and consistent determination and use of diagnosis, estimated infection, and/or estimated seroconversion dates. For advancing the greater HIV prevention research agenda, early negotiation of data sharing agreements among study teams would be ideal.

Recommendations: study design and operational details

An important consideration when planning to follow seroconverters is to determine how long to follow them within the context of the parent study and when to ask them to enroll in a seroconverter rollover study. The timing of transition into a rollover study impacts the number of participants recruited and long-term retention. Increasing retention (i.e., the proportion of all seroconverters in a parent study that roll over into the seroconverter study) is crucial to obtain sufficient sample size for comparative analysis. There are presently insufficient data on retention because many parent studies are ongoing and remain blinded, so it is premature to recommend an optimal time point for transition from parent to rollover studies. However, anecdotally, retention is higher and easier when seroconverters are followed within the context of the parent study for some period of time. Consent for remaining in an HIV prevention study in the event of seroconversion can be obtained from all participants at the beginning of the parent study, but consent for enrollment in a long-term seroconverter follow-up study must be obtained separately. Also, follow-up visits specific to seroconverters can be coordinated with regular study visits, making it more convenient for the participants and further promoting retention.

Following seroconverters within the context of the parent studies for a limited period also facilitates the capture of critical early postdiagnosis time point data; early time points, evaluations, and events may pertain to the objectives of the parent study. The MTN-015 study team reported that enrollment delays have occurred while HIV diagnoses are confirmed. Therefore, the MTN and other groups incorporated collection of early postdiagnosis samples into the parent protocols. Another strategy for increasing retention in both the parent study at the time of HIV diagnosis and enrollment into a rollover study is to have a dedicated team of counselors meet with participants who are diagnosed with HIV infection, as was done during the CAPRISA 004 study when participants were asked to enroll in Tenofovir gel Research for Advancing Prevention Science (TRAPS). HIV diagnosis is a difficult and challenging time both for the participants and the counselors/clinicians who work with them. Counseling by a trained, dedicated team provides needed support and encouragement to remain on-study.

Building short-term seroconverter follow-up into the parent study promotes retention at the point of HIV diagnosis, allows time for obtaining consent for the rollover study, and facilitates collection of clinical data and specimens during early postdiagnosis time points, but long-term follow-up of seroconverters is costly and difficult to budget. Enrolling seroconverters from multiple parent studies into a single or a few seroconverter rollover studies may be more efficient and logistically feasible. On the basis of these considerations the SSG makes the protocol development, implementation, and timing recommendations in Table 5. When developing a seroconverter rollover study and determining the optimal point of enrollment transition, the desire to ensure retention and capture early time points postdiagnosis has to be weighed against any potential gain in efficiency and cost savings of a rollover study.

Duration of follow-up postdiagnosis would need to vary by necessity, depending on the objectives of the parent and rollover studies. For example, questions regarding acute infection and correlates of harm and protection would require a short duration of follow-up, perhaps until participants reach viral set point. Those studies looking at disease progressions might follow participants until initiation of treatment. Studies looking at response to treatment or comorbidities such as chronic inflammation would necessitate a longer duration of follow-up. To mitigate the costs of an extended follow-up period, the frequency of visits would decrease over time. Potentially, novel and inexpensive point-of-care testing technologies could be incorporated to save cost and decrease burden on participants and clinics.

Recommendations: study objectives

The studies were compared to determine the common study objectives that would require follow-up of seroconverters, and to recommend a core set of these objectives for inclusion in all protocols that follow seroconverters (both parent and rollover studies). Delineation of a core set of objectives would facilitate protocol development and future cross-study analyses of clinical and laboratory data. The SSG found there were a few objectives that were common to many studies, and

Table 5. Summary of recommendations.

Component	Recommendations
Development and implementation of seroconverter studies	Develop network-specific or cross-network seroconverter studies for the long-term follow-up of study participants who seroconvert while participating in a network prevention study
Timing of enrollment into seroconverter rollover studies	Follow seroconverters in the short-term with in the context of the parent study before or concurrent with rollover into a seroconverter follow-up study Obtain consent for short-term and long-term follow-up postseroconversion, including the collection of samples for future genomic testing, at the time of enrollment
Study objectives	Develop for all prevention trials and all seroconverter rollover studies a core set of objectives; allow study teams to add study-specific objectives as necessary. These objectives should include: Describe/compare the virological, immunological, and clinical course of HIV disease among seroconverters in active compared to control arm Assess drug resistance Compare changes in risk behavior pre and postseroconversion Evaluate response to therapy Bank specimens in ample volumes for future assays, including genomic testing
Other recommendations for enhanced standardization of data collection	Standardize definition, determination, and use of diagnosis dates, estimated infection date, and estimated seroconversion date
Sample collections, biological assays, assessments and repository development	Develop a core set of sample collections and assessments for all prevention trials and all seroconverter rollover studies; allow study teams to add study-specific sample collections and assessments as necessary. Examples might include:

Samples and assessments	Presxn:	0–3 months Postsxn	6–12 months Postsxn	>12 months Postsxn
HIV rapid test	M	–	–	–
Urine/pregnancy test (women only)	M	M	Q	S
Clinical assessment	M	M	Q	S
Behavioral assessment	M	M	Q	S
Blood for HIV RNA testing and storage	Q	M	Q	S

M, Monthly; Q, Quarterly; S, Semiannually; sxn, seroconversion.

also identified some objectives that are currently included in two to four studies, but whose inclusion in future studies would be recommended (see Table 5).

The most common objectives shared across all protocols were to describe and compare the virological, immunological, and clinical course of HIV disease among seroconverters in active and placebo arms and to assess drug resistance; these objectives are included in the core set of recommended objectives. Although most studies collect behavioral risk information, very few have objectives related to behavioral risk. Although these objectives are not common, they would be recommended for inclusion in a core set of objectives. Similarly, although development of a specimen repository is a specific objective in only four studies, it is recommended as one of the core objectives. An evaluation of response to ART is also recommended, as selected drug resistance (owing to oral or topical antiretroviral-based PrEP), or disease progression modulation (via vaccine induction), may impact the natural history of treated and untreated disease. There were many objectives that were common to only a small number of studies (one to three), but would not be suitable for inclusion in a core set of objectives. These objectives would be considered to be study-specific, and are examples of objectives that a protocol team might choose to include in addition to the recommended core objectives as appropriate (see Supplemental Digital Content 1 and 2, http://links.lww.com/QAD/A297, detailed objectives of each protocol).

One might expect differences between vaccine and PrEP (microbicide and oral) protocols, but many similarities were found, indicating that a core set of seroconverter-related objectives could be used in vaccine and PrEP studies. Furthermore, as uptake of oral PrEP increases and vaccine or other biomedical prevention studies allow for or provide PrEP, rollover studies that include participants from vaccine studies may need to evaluate the effects of PrEP on seroconversion and other postseroconversion endpoints.

Recommendations: sample collections and assessments

To facilitate analysis of clinical and laboratory data across studies, the protocols were compared to determine the common sample collections/assessments and to develop a core set that would be recommended for inclusion in all studies that follow seroconverters. We found that the samples collected varied across protocols, but those supporting the common objectives described above (whole blood, serum/plasma, and PBMCs), urine for pregnancy testing, and various genital secretions and/or biopsies are collected for most studies (Table 4) and would be recommended for inclusion in a core set of samples.

Behavioral assessments are collected in most studies both before and after diagnosis, but will pose particular challenges to cross-protocol analysis. The specific behavioral assessments, time frame for reporting, method of administration, and scales all vary across protocols, impeding cross-protocol comparison. Although acknowledging that no gold standard exists for behavioral assessments, development of best practices would facilitate both cross-protocol comparison and development of future protocols.

Ideally, specimens (e.g. blood, cells, and genital secretions) should be banked in ample volumes for future assays. Although only three rollover protocols (MTN-015, HVTN 403, and HVTN 404) have specific study objectives for developing a repository of specimens for future analysis, most of the protocols store samples for future testing at the site or at a central repository. Most commonly stored are serum/plasma and cryopreserved PBMCs; collection and repository storage of these samples is recommended. Acknowledging that storage of such specimens is particularly costly and poses additional challenges in international settings, such a repository would be high-yield for answering both current and future research questions, many of which may be exploratory and/or not fully developed at the time of protocol conduct. Collection and storage of other sample types could be added to meet study-specific objectives.

Maximum blood draw volumes varied widely, ranging from 20 to 250 ml. It is unclear what factors contributed to this range, but reports from study teams suggested that local restrictions, cultural norms, and ethical considerations may be a limiting factor. It has been reported that some African communities believe a loss of blood leads to a loss of energy, immune function, and/or virility, or that it might be used in magic [44–47]. Culturally sensitive training for site staff and participants may be required to support collection of blood in volumes sufficient for real-time and future testing,

Some investigators report that there have been challenges to obtaining open-ended approval for genomic studies from national/local ethics committees, which may require more specific analysis plans and details for future testing than are typically needed for regulatory approval at US domestic sites. Ideally, collection of genomic information would be included in a core set of sample collections/assessments, although regulatory requirements may prove to be challenging.

Standardized timing of sample collections/assessments in various protocols must also be considered if meaningful conclusions are to be drawn, especially for early infection events. For follow-up schedules and analysis, postseroconversion time lines should be defined consistently and studies should be explicit in what primary data were acquired (e.g. diagnosis date, estimated infection date, estimated seroconversion date). Diagnosis is the most feasible trigger for postseroconversion follow-up, but depending on the frequency of testing, diagnosis could occur at different phases of actual infection, leading to impacts on analyses of timing of early infection events, for which estimated infection and seroconversion dates may be more useful.

Consistent definitions, determinations and uses of diagnosis, estimated infection, and estimated seroconversion dates will depend on a common frequency of rapid HIV testing and storage of plasma/blood samples across parent studies. To more accurately estimate dates of infection and seroconversion, plasma samples or dried blood spots could be stored from all visits in case HIV RNA testing is needed at a later date. Such an approach was taken in FEM-PrEP, which used monthly antibody testing and collection of plasma, and HVTN 505, which employed antibody testing and storage of blood samples less frequently. During protocol development, the cost of collecting and storing plasma/blood samples for possible HIV RNA analysis would need to be balanced against the analytical needs.

Frequent (monthly) HIV testing is advantageous, facilitating an accurate estimation of seroconversion and/or infection dates and collection of samples/assessments at early time points. Furthermore, although study participants are counseled to be alert for signs of acute infection syndrome, few seroconverters are identified by clinical symptoms [48]. Most seroconverters do experience clinical symptoms attributable to acute HIV infection, but they do not always realize or report it [49]. Therefore, frequent HIV testing also has the advantage of identifying more participants during acute infection. Also, HIV testing reduces the potential for prolonged exposure to incompletely suppressive ART in the face of occult/undetected acute/primary HIV infection. We found that HIV antibody testing is usually done on a monthly or quarterly basis; testing less frequently than monthly not only complicates estimation of seroconversion date but could also increase seroconverters' exposure to suboptimal treatment regimens. However, the cost and feasibility of monthly testing must also be considered. The CDC's interim guidance for the use of PrEP for prevention of HIV infection in MSM now calls for testing every 2–3 months [50] and some upcoming studies, such as HPTN 069 [37], will use quarterly testing to reduce costs. Although monthly testing for HIV is costly, new testing technologies (e.g., combined antibody/antigen tests) might make it more feasible. Until then, the advantages of frequent HIV testing must be balanced against its costs and participant acceptability of such frequent assessments. Protocol teams might adjust the frequency of HIV testing to the intervention method. For example, vaccine studies in which participants are not taking PrEP might test quarterly; studies in which participants do take PrEP might reduce risk of prolonged exposure to suboptimal treatments by using monthly testing.

Although considerable overlap exists among protocols in regard to sample collections/assessments, variation in timing complicates comparison across protocols. To more easily and effectively aggregate and compare findings from multiple protocols, the SSG makes the recommendations and provides examples in Table 5. These examples are based on a hypothetical harmonization of current and recent protocols. Design of a collaborative rollover protocol would require careful consideration of the study

objectives, the necessary samples/assessments, and the available resources.

Owing to a low rate of seroconversion in prevention studies and the need to follow seroconverters long term to address present research questions, it would be advantageous to consider cross-sponsor data utilization agreements that would allow aggregation of data from multiple studies, and promote data usage by a broad research community. Aggregation of data can be made possible by a common seroconverter rollover protocol or protocol design that includes a core set of study objectives, samples, and assessments. Common standards for consent and retention approaches and a core set of objectives, samples and assessments would require commitment and coordination across research teams. Study-specific goals could be accommodated by allowing study teams to include additional objectives, sample collections, and assessments as needed.

Acknowledgements

The SSG would like to thank Lut Van Damme of Family Health International 360 and Robert O'Connell of the Military HIV Research Program for joining discussions of FEM-PrEP and RV144/RV152, respectively, and Niles Eaton of the HVTN for addressing questions regarding the HVTN protocols.

P.E. contributed to primary acquisition of data, analysis and interpretation of data, drafting of article, and final approval. R.L. contributed to concept and design; acquisition, analysis, and interpretation of data; critical review of article; final approval. S.S. contributed to concept and design; acquisition, analysis, and interpretation of data; critical review of article; final approval. M.E.S. contributed to concept and design; acquisition, analysis, and interpretation of data; critical review of article; final approval. S.A.R. contributed to concept and design; acquisition, analysis, and interpretation of data; critical review of article; final approval. C.K. contributed to concept and design; acquisition, analysis, and interpretation of data; critical review of article; final approval. A.T. contributed to concept and design; acquisition, analysis, and interpretation of data; critical review of article; final approval. J.S. contributed to concept and design; acquisition, analysis, and interpretation of data; critical review of article; final approval.

Conflicts of interest

P.E. and J.S. are currently receiving a grant (U01 A1068614) from the National Institute of Health/ National Institute of Allergy and Infectious Diseases (NIH/NIAID). R.L. is working with Gilead Sciences as a site Principal Investigator for a multisite treatment study, receiving drug for two prevention protocols and currently receiving a grant (K23DA026308) from the NIH. S.S. represents CAPRISA studies that received funding from the US Agency for International Development, Family Health International 360, LIFElab, and the Centre for the AIDS Programme of Research in South Africa (cooperative grant # GPO-A-00–05–00022–00, contract # 132119), and CONRAD (cooperative grant: GP00–08–00005–00, subproject agreement # PPA-09–046O). M.S. is currently receiving a grant (U01-AI069470) from NIH/NIAID, and this publication was supported by the National Center for Advancing Translational Sciences, NIH, through Grant Number UL1 TR000040, formerly the National Center for Research Resources, Grant Number UL1 RR024156. S.R. is currently receiving a grant (5 UM1 AI068633) from NIH/NIAID. A.T. is a consultant with Merck and currently receiving a grant (subproject from U01AI034994) from NIH/NIAID. C.K. is receiving a grant (5 UM1 AIO68614) from NIH/NIAID.

References

1. del Amo J, del Romero J, Barrasa A, Perez-Hoyos S, Rodriguez C, Diez M, *et al*. **Factors influencing HIV progression in a seroconverter cohort in Madrid from 1985 to 1999.** *Sex Transm Infect* 2002; **78**:255–260.
2. Hecht FM, Wang L, Collier A, Little S, Markowitz M, Margolick J, *et al*. **A multicenter observational study of the potential benefits of initiating combination antiretroviral therapy during acute HIV infection.** *J Infect Dis* 2006; **194**:725–733.
3. Taylor JM, Schwartz K, Detels R. **The time from infection with human immunodeficiency virus (HIV) to the onset of AIDS.** *J Infect Dis* 1986; **154**:694–697.
4. Hessol NA, Koblin BA, van Griensven GJ, Bacchetti P, Liu JY, Stevens CE, *et al*. **Progression of human immunodeficiency virus type 1 (HIV-1) infection among homosexual men in hepatitis B vaccine trial cohorts in Amsterdam, New York City, and San Francisco, 1978–1991.** *Am J Epidemiol* 1994; **139**:1077–1087.
5. Cozzi Lepri A, Pezzotti P, Dorrucci M, Phillips AN, Rezza G. **HIV disease progression in 854 women and men infected through injecting drug use and heterosexual sex and followed for up to nine years from seroconversion. Italian Seroconversion Study.** *BMJ* 1994; **309**:1537–1542.
6. Kahn JO, Walker BD. **Acute human immunodeficiency virus type 1 infection.** *N Engl J Med* 1998; **339**:33–39.
7. Pilcher CD, Joaki G, Hoffman IF, Martinson FE, Mapanje C, Stewart PW, *et al*. **Amplified transmission of HIV-1: comparison of HIV-1 concentrations in semen and blood during acute and chronic infection.** *AIDS* 2007; **21**:1723–1730.
8. Emmons CA, Joseph JG, Kessler RC, Wortman CB, Montgomery SB, Ostrow DG. **Psychosocial predictors of reported behavior change in homosexual men at risk for AIDS.** *Health Educ Q* 1986; **13**:331–345.
9. Chmiel JS, Detels R, Kaslow RA, Van Raden M, Kingsley LA, Brookmeyer R. **Factors associated with prevalent human immunodeficiency virus (HIV) infection in the Multicenter AIDS Cohort Study.** *Am J Epidemiol* 1987; **126**:568–577.
10. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, *et al*. **Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection.** *Cell* 1996; **86**:367–377.
11. Lambotte O, Boufassa F, Madec Y, Nguyen A, Goujard C, Meyer L, *et al*. **HIV controllers: a homogeneous group of HIV-1-infected patients with spontaneous control of viral replication.** *Clin Infect Dis* 2005; **41**:1053–1056.
12. Egger M, Hirschel B, Francioli P, Sudre P, Wirz M, Flepp M, *et al*. **Impact of new antiretroviral combination therapies in HIV infected patients in Switzerland: prospective multicentre study. Swiss HIV Cohort Study.** *BMJ* 1997; **315**:1194–1199.

13. Colfax GN, Buchbinder SP, Cornelisse PG, Vittinghoff E, Mayer K, Celum C. **Sexual risk behaviors and implications for secondary HIV transmission during and after HIV seroconversion.** *AIDS* 2002; **16**:1529–1535.
14. Fox R, Odaka NJ, Brookmeyer R, Polk BF. **Effect of HIV antibody disclosure on subsequent sexual activity in homosexual men.** *AIDS* 1987; **1**:241–246.
15. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. **Acute HIV-1 Infection.** *N Engl J Med* 2011; **364**:1943–1954.
16. Sailasuta N, Ananworanich J, Chalermchai T, DeGruttola V, Lerdlum S, Pothisri M, *et al.* **Brain tCho/Cr is elevated in acute HIV within the first month of infection [456]**. In: *19th Conference on Retroviruses and Opportunistic Infections 2012*. Seattle.
17. van Loggerenberg F, Mlisana K, Williamson C, Auld SC, Morris L, Gray CM, *et al.* **Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 acute infection study.** *PLoS One* 2008; **3**:e1954.
18. Kibuuka H, Rono K, Maganga L, Millard M, Sekiziyivu A, Maboko L, *et al.* **Pattern of HIV risk behavior in a cohort of high risk women in East Africa.** *Retrovirology* 2012; **9 (Suppl 2)**:124.
19. Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, Li H, *et al.* **Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection.** *J Exp Med* 2009; **206**:1273–1289.
20. Zhu T, Wang N, Carr A, Nam DS, Moor-Jankowski R, Cooper DA, *et al.* **Genetic characterization of human immunodeficiency virus type 1 in blood and genital secretions: evidence for viral compartmentalization and selection during sexual transmission.** *J Virol* 1996; **70**:3098–3107.
21. Buchacz K, Hu DJ, Vanichseni S, Mock PA, Chaowanachan T, Srisuwanvilai LO, *et al.* **Early markers of HIV-1 disease progression in a prospective cohort of seroconverters in Bangkok, Thailand: implications for vaccine trials.** *J Acquir Immune Defic Syndr* 2004; **36**:853–860.
22. Hubert JB, Burgard M, Dussaix E, Tamalet C, Deveau C, Le Chenadec J, *et al.* **Natural history of serum HIV-1 RNA levels in 330 patients with a known date of infection. The SEROCO Study Group.** *AIDS* 2000; **14**:123–131.
23. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. **Prognosis in HIV-1 infection predicted by the quantity of virus in plasma.** *Science* 1996; **272**:1167–1170.
24. Langford SE, Ananworanich J, Cooper DA. **Predictors of disease progression in HIV infection: a review.** *AIDS Res Ther* 2007; **4**:11.
25. Cohen MS, McCauley M, Gamble TR. **HIV treatment as prevention and HPTN 052.** *Curr Opin HIV AIDS* 2012; **7**:99–105.
26. Celentano D, Chariyalertsak S, Chingono A, Coates TJ, Donnell D, Gray G, *et al.* **Project Accept (HPTN 043): a phase III randomized controlled trial of community mobilization, mobile testing, same-day results, and post-test support for HIV in Sub-Saharan Africa and Thailand.** *HIV Prevention Trials Network*. http://www.cbvct.med.ucla.edu/protocol.pdf. [Accessed 2 January 2013].
27. Pettifor A. **HPTN 068: Effects of cash transfer for the prevention of HIV in young South African women**. *HIV Prevention Trials Network*. http://www.hptn.org/web%20documents/HPTN068/ProtocolVersions/HPTN068v1_06Oct2010.pdf. [Accessed 2 January 2013].
28. Reilly L. **Follow-up study to RV144 HIV vaccine trial shows no effect on disease progression in infected volunteers**. *U.S. Military HIV Research Program*. http://www.hivresearch.org/news.php?NewsID=220. [Accessed 2 January 2013].
29. Van Damme L, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, *et al.* **Preexposure prophylaxis for HIV infection among African women.** *N Engl J Med* 2012; **367**:411–422.
30. Connick E, MaWhinney S, Wilson CC, Campbell TB. **Challenges in the study of patients with HIV type 1 seroconversion.** *Clin Infect Dis* 2005; **40**:1355–1357.
31. Schwab E. **A study of patients who develop HIV infection after enrolling in HIV vaccine trials or HIV vaccine preparedness trials**. *ClinicalTrials.gov*. http://clinicaltrials.gov/ct2/show/NCT00029913. [Accessed 2 January 2013].
32. Newman R. **What's happening with HVTN 404 & HVTN 802?** The CAB Bulletin. *HIV Vaccine Trials Network* 2009; **10**:1,7.
33. Riddler SA. **MTN-015: an observational cohort study of women following HIV-1 seroconversion in microbicide trials, version 1.0**. *Microbicides Trials Network*. http://www.mtnstopshiv.org/sites/default/files/attachments/v1.0_final_19jun07.pdf. [Accessed 2 January 2013].
34. HPTN. **HPTN 067 study specific procedures manual, V2.0.** *HIV Prevention Trials Network*. http://www.hptn.org/web%20documents/HPTN067/SSP/Ver2/067SSP_FrontSectionV2.pdf. [Accessed 2 January 2013].
35. Chirenje ZM, Marrazzo J. **MTN-003: phase 2B safety and effectiveness study of tenofovir 1% gel, tenofovir disoproxil fumarate tablet and emtricitabine/tenofovir disoproxil fumarate tablet for the prevention of HIV infection in women, version 2.0.** *Microbicides Trials Network*. http://www.mtnstopshiv.org/sites/default/files/attachments/MTN-003_FINAL_Version_2.0_31DEC2010.pdf. [Accessed 2 January 2013].
36. Grant RM, van Griensven F. **HPTN 067: the ADAPT study, version 3.0**. *HIV Prevention Trials Network*. http://www.hptn.org/web%20documents/HPTN067/Protocol/067ProtocolV3_01Dec2011.pdf. [Accessed 2 January 2013].
37. Gulick RM, Mayer K, Wilkin T. **HPTN 069: NEXT-PREP: novel exploration of therapeutics for PREP, version 2.0**. *HIV Prevention Trials Network*. http://www.hptn.org/web%20documents/HPTN069/StudyDocs/HPTN069V2_09April2012.pdf. [Accessed 2 January 2013].
38. Hammer S, Sobieszczyk M, Yin M. **Safety and effectiveness of HIV-1 DNA plasmid vaccine and HIV-1 recombinant adenoviral vector vaccine in HIV-uninfected, circumcised men and male-to-female (MTF) transgender persons who have sex with men**. *HIV Vaccine Trials Network*. http://clinicaltrials.gov/show/NCT00865566. [Accessed 2 January 2013].
39. Valley-Omar Z, Sibeko S, Anderson J, Goodier S, Werner L, Arney L, *et al.* **CAPRISA 004 tenofovir microbicide trial: no impact of tenofovir gel on the HIV transmission bottleneck**. *J Infect Dis* 2012; **206**:35–40.
40. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, *et al.* **Preexposure chemoprophylaxis for HIV prevention in men who have sex with men.** *N Engl J Med* 2010; **363**:2587–2599.
41. Bangsberg D, Haberer J, Psaros C, Baeten J, Katabira E, Tumwesigye E, *et al.* **High adherence and high effectiveness observed in HIV discordant couples: partners PrEP study, adherence monitoring and counseling sub-study [1067]**. In: *19th Conference on Retroviruses and Opportunistic Infections, 2012*. Seattle.
42. van Damme L, Cornel A, Ahmed K, Agot K, Lombaard J, Kapiga S, *et al.* **The FEM-PrEP trial of emtricitabine/tenofovir disoproxil fumarate (truvada) among African Women [32LB]**. In: *19th Conference on Retroviruses and Opportunistic Infections, 2012*. Seattle.
43. Grant RM, Lama JR. **Emtricitabine/tenofovir disoproxil fumarate for HIV prevention in men**. *National Institute of Allergy and Infectious Diseases*. http://clinicaltrials.gov/show/NCT00458393. [Accessed 2 January 2013].
44. Fairhead J, Leach M, Small M. **Where techno-science meets poverty: Medical research and the economy of blood in The Gambia, West Africa.** *Soc Sci Med* 2006; **63**:1109–1120.
45. Geissler PW. **'Kachinja Are Coming!': encounters around medical research work in a Kenyan village.** *Africa* 2005; **75**:173–202.
46. Olaiya MA, Alakija W, Ajala A, Olatunji RO. **Knowledge, attitudes, beliefs and motivations towards blood donations among blood donors in Lagos, Nigeria.** *Transfus Med* 2004; **14**:13–17.
47. Ottong JG, Asuquo EEJ, Olaniran NS, Duke FD, Abia RP. **Community mobilization for blood donation, Cross River State, Nigeria.** *Int J Gynecol; Obstet* 1997; **59 (Suppl 2)**:S119–S125.
48. Celum CL, Buchbinder SP, Donnell D, Douglas JM Jr, Mayer K, Koblin B, *et al.* **Early human immunodeficiency virus (HIV) infection in the HIV Network for Prevention Trials Vaccine Preparedness Cohort: risk behaviors, symptoms, and early plasma and genital tract virus load.** *J Infect Dis* 2001; **183**:23–35.
49. Schacker T, Collier AC, Hughes J, Shea T, Corey L. **Clinical and epidemiologic features of primary HIV infection.** *Ann Intern Med* 1996; **125**:257–264.
50. Smith DK, Grant RM, Weidle PJ, Lansky A, Mermin J, Fenton KA, *et al.* **Morbidity and Mortality Weekly Report (MMWR): interim guidance: preexposure prophylaxis for the prevention of HIV infection in men who have sex with men**. *Centers for Disease Control and Prevention*. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6003a1.htm. [Accessed 2 January 2013].