Appendix 1, Protocol

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Background:

HIV-2 infection is less pathogenic than HIV-1. Both viruses are prevalent in West Africa, and dual infections with HIV-1 and HIV-2 (HIV-D) are relatively common in that region [1, 2]. The mortality rate (MR) among HIV-1-monoinfected individuals is substantially higher than the rate among HIV-2-monoinfected individuals [3, 4]. Previous research suggested that HIV-2 infection inhibits HIV-1 disease progression in dually infected individuals [5-9], whereas other studies were not in accord [10, 11]. Whether the MR of HIV-D-infected individuals is as high as the MR of those with HIV-1 or whether it is lower is an ongoing debate [2, 5, 12, 13].

Objective:

Provide an overview of the current knowledge about mortality rates of HIV-1/HIV-2 dual (HIV-D)-infected individuals and HIV-1- or HIV-2-monoinfected individuals.

Research questions:

1) Do people infected with HIV-D have a different mortality rate compared with people infected with only HIV-1?
2) Do people infected with HIV-D have a different mortality rate compared with people infected with only HIV-2?
3) Do people infected with only HIV-1 have a different mortality rate compared with people infected with only HIV-2?

Participants:

The study population should include at least two HIV groups (i.e., HIV-D and HIV-1, HIV-D and HIV-2, or HIV-1 and HIV-2). No further selection criteria should be used; co-morbidities are not an exclusion criterion.

Outcome:

The outcome is mortality. The main outcome measure we will consider is the mortality rate, and the main effect measure will be the mortality rate ratio (MRR).

Study design:

Systematic review and meta-analysis of longitudinal studies.

Language:

We intend to include all languages.

Search strategy:

Medline and EMBASE databases will be searched using the words HIV-1, HIV-2, mortality, death, fatality, survival, disease progression, outcome assessment, and their MeSH terms, as listed below. Reference lists of articles included in the full-text screening will be searched manually.

The following search strategy will be used in Medline:

(("HIV-1"[Mesh] OR HIV-1*[tiab] OR HIV1*[tiab] OR HIV type 1[tiab] OR human immunodeficiency virus 1[tiab] OR human immunodeficiency virus type 1[tiab]) AND ("HIV-2"[Mesh] OR HIV-2*[tiab] OR HIV2*[tiab] OR HIV type 2[tiab] OR human immunodeficiency virus 2[tiab] OR human immunodeficiency virus type 2[tiab])) AND (("Mortality"[Mesh] OR "mortality"[Subheading] OR mortalit*[tiab]) OR ("Survival Rate"[Mesh] OR surviv*[tiab]) OR ("Death"[Mesh] OR death*[tiab]) OR ("Outcome Assessment (Health Care)"[Mesh] OR outcome*[tiab]) OR (fatalit*[tiab]) OR ("Disease Progression"[Mesh] OR disease progression[tiab]))

The following search strategy will be used in EMBASE:

1. exp Human immunodeficiency virus 1/
2. (hiv-1 or hiv1 or hiv type 1 or human immunodeficiency virus 1 or human immunodeficiency virus type 1).ti,ab.
3. 1 or 2
4. exp Human immunodeficiency virus 2/
5. (hiv-2 or hiv2 or hiv type 2 or human immunodeficiency virus 2 or human immunodeficiency virus type 2).ti,ab.
6. 4 or 5
7. 3 and 6
8. exp mortality/

9. mortalit*.ti,ab.
10. exp survival/
11. surviv*.ti,ab.
12. exp death/
13. death*.ti,ab.
14. exp outcome assessment/
15. outcome*.ti,ab.
16. fatalit*.ti,ab.
17. disease course/
18. disease progression.ti,ab.
19. 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18
20. 7 and 19

Study selection:

Study selection will be performed by two independent researchers who will screen titles, abstracts and full texts. Any disagreement will be solved by consensus. If consensus is not reached, a third researcher will make the final decision.

Data extraction:

Data will be extracted by three independent researchers. Any disagreement will be solved by consensus. If consensus is not reached, a fourth researcher will make the final decision. If data are missing, authors of primary studies will be contacted to provide missing or additional data. A list of data that will be extracted is shown in table A.1.

Quality assessment:

To assess the risk of bias, we will develop a quality assessment checklist. This checklist will be based on the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) checklist [14] and the Newcastle Ottawa checklist [15] and adjusted specifically for this review. The checklist is shown in table A.2. Checklist items will be scored by three independent researchers using +, ±, -. Differences will be solved by consensus. If consensus is not reached, a fourth researcher will make the decision.

Data analysis:

We will perform a meta-analysis with random-effects models. The outcomes of the analyses are the mortality rate ratio (MRR) of HIV-D versus HIV-1 infection, the MRR of HIV-D versus HIV-2 infection, and the MRR of HIV-1 versus HIV-2 infection.

If there are differences in disease progression and mortality between HIV groups, they will be more easily detected in cohorts of asymptomatic individuals than in cohorts with advanced disease [12, 16]. To account for this, we will perform two a priori defined subanalyses. In subanalysis 1, we will estimate the MRR by patient setting (i.e., community versus hospital/clinic). In subanalysis 2, we will estimate the MRR by stage of disease of the population (i.e., recent HIV-infected populations versus populations with a more advanced stage of disease). The stage of HIV infection will be determined on the basis of such factors as reported time of infection, CD4 counts, age, co-morbidities, and AIDS diagnosis. In subanalysis 3, we will estimate the MRR by quality of the study (i.e. low versus high quality), as the quality might influence the outcome.

Heterogeneity will be explored by estimating the measure of inconsistency (I^2). The definition of I^2 is the percentage of total variability in a set of effect sizes due to true heterogeneity or between-studies variability [17]. We consider I^2<35% as low, 35≥ I^2≤ 65% as moderate and I^2>65% as high. If there is a considerable degree of heterogeneity, we will perform a leave-one-out sensitivity analysis, and if the number of included studies exceeds 10, we will explore the data in subgroups or perform a meta-regression analysis to understand the reasons for heterogeneity.

To examine possible publication bias, we will create funnel plots and test for asymmetry when at least 10 studies are included [18].

Manuscript preparation:

In writing the manuscript, we will use the Preferred Reporting Items for Systematic Reviews and Meta-Analyses（PRISMA) statement [19].

Table A.1. Data extraction list

Article information		
1	Author	Family name of first author and first initial
2	Title	Title of the article
3	Publication	Journal & year of publication
4	Country	Country where research was done
Exposure		
5	Prevalent/incident HIV-1	Were the HIV-1 cases prevalent or incident in the study period? Incident cases are defined as individuals for whom the approximate date of seroconversion is known.
6	Prevalent/incident HIV-2	Were the HIV-2 cases prevalent or incident in the study period? Incident cases are defined as individuals for whom the approximate date of seroconversion is known.
7	Prevalent/incident HIV-D	Were the HIV-D cases prevalent or incident in the study period? Incident cases are defined as individuals of whom the approximate date of seroconversion is known.
8	Diagnostics	Which diagnostic methods were used for the testing of HIV? Specify the methods used. If tests were done multiple times, or different tests were used, describe this.
Study population characteristics		
9	Inclusion criteria	Describe the inclusion criteria that were used in the study.
10	Start inclusion	Date of beginning of the inclusion period (month-year).
11	Stop inclusion	Date of end of the inclusion period (month-year).
12	Start FU	Date of beginning of the FU period (month-year).
13	Stop FU	Date of end of the FU period (month-year).
14	Maximum FU	What was the maximum FU according to the study design?
15	FU methods	How were patients followed? Clinic visits by patients or home visits by researchers? What was the planned frequency of

		visits? How was it established whether patients had died? What were the FU conditions?
16	Source	Where were the individuals selected: from patients attending a hospital; from the community; from an occupational cohort?
17	Source name	Name the hospital or clinic and city from where the patients were selected.
18	General population characteristics	Describe the characteristics of the study population, such as co-morbidities, ethnicity etc.
19	Age range	If shown in the paper, the full range of age and the mean and 95% CI or median and IQR. Present numbers only for the population that is used in the mortality analyses.
20	Gender	How many males/females were included? Present as M:200 F:150 (200 men, 150 women). If numbers are given for the different HIV-groups and mortality, also report these stratified numbers under the corresponding subheadings. Present numbers only for the population that is used in the mortality analyses.
21	Co-morbidity	If reported, what co-morbidities did study participants have? Were the co-morbidities present at inclusion or were they incident during the study period, describe time period. Did the study participants receive treatment for these? What % or number of patients had the co-morbidity?
22	ART	Were the individuals treated with antiretroviral therapy during the study period? If so, were they censored in the mortality analysis from date of start of ART, or were they excluded from the study? Provide the information for HIV-1, HIV-2 and HIV-D groups.
23	Co-trimoxazole prophylaxis	Were individuals provided with co-trimoxazole prophylaxis? Provide the information for HIV-1, HIV-2 and HIV-D groups.
24	CD4 count HIV-1	If CD4 counts data are given, write down how many HIV-1 infected persons belonged to each CD4 category that was given.
25	CD4 % HIV-1	If CD4% data are given, write down how many HIV-1 infected persons belonged to each CD4 category that was given.
26	CD4 count HIV-2	If CD4 counts data are given, write down how many HIV-2 infected persons belonged to each CD4 category that was given.
27	CD4 % HIV-2	If CD4% data are given, write down how many HIV-2 infected persons belonged to each CD4 category that was given.
28	CD4 count HIV-D	If CD4 counts data are given, write down how many HIV-D infected persons belonged to each CD4 category that was given.
29	CD4 % HIV-D	If CD4% data are given, write down how many HIV-D infected persons belonged to each CD4 category that was given
30	Viral load HIV-1	If viral loads data are available, write down how many HIV-1 infected persons belonged to each viral load group/range that was given.

31	Viral load HIV-2	If viral loads data are available, write down how many HIV-2 infected persons belonged to each viral load group/range that was given.
32	Viral load HIV-1 in HIV-D	Provide the load for HIV-1. If viral loads data are available, write down how many HIV-D infected persons belonged to each viral load group/range that was given
33	Viral load HIV-2 in HIV-D	Provide the load for HIV-2. If viral loads data are available, write down how many HIV-D infected persons belonged to each viral load group/range that was given.
Raw data		
34	HIV-1 total	How many HIV-1 infected individuals are included in the mortality analysis?
35	HIV-2 total	How many HIV-2 infected individuals are included in the mortality analysis?
36	HIV-D total	How many HIV-D infected individuals are included in the mortality analysis?
37	Pyo-1	What was the FU time in person-years of observation of HIV-1 infected people?
38	Pyo-2	What was the FU time in person-years of observation of HIV-2 infected people?
39	Pyo-D	What was the FU time in person-years of observation of HIV-D infected people?
40	HIV-1 died	How many HIV-1 infected individuals died during the study period?
41	HIV-2 died	How many HIV-2 infected individuals died during the study period?
42	HIV-D died	How many HIV-D infected individuals died during the study period?
43	Lost to FU HIV-1	How many individuals, who were HIV-1 infected, were lost during FU?
44	Lost to FU HIV-2	How many individuals, who were HIV-2 infected, were lost during FU?
45	Lost to FU HIV-D	How many individuals, who were HIV-D infected, were lost during FU?
Crude and adjusted estimates		
46	Mortality rate-1	What was the mortality rate (unit: $[100\ PY]^{-1}$) among HIV-1 infected people?
47	Mortality rate-2	What was the mortality rate (unit: $[100\ PY]^{-1}$) among HIV-2 infected people?
48	Mortality rate-D	What was the mortality rate (unit: $[100\ PY]^{-1}$) among HIV-D infected people?
49	Mortality rate ratio-D1	Crude mortality rate ratio or hazard ratio, comparing HIV-D infected to HIV-1 infected individuals. Only report if given in the

		paper (don't calculate yourself). Define whether the MRR or HR is given
50	Mortality rate ratio-D2	Crude mortality rate ratio or hazard ratio, comparing HIV-D infected to HIV-2 infected individuals. Only report if given in the paper (don't calculate yourself). Define whether the MRR or HR is given
51	Mortality rate ratio-12	Crude mortality rate ratio or hazard ratio, comparing HIV-1 infected to HIV-2 infected individuals. Only report if given in the paper (don't calculate yourself). Define whether the MRR or HR is given
52	Mortality rate ratio-D1 adjusted	Adjusted mortality rate ratio or hazard ratio, comparing HIV-D infected to HIV-1 infected individuals. Define whether the MRR or HR is given.
53	Mortality rate ratio-D2 adjusted	Adjusted mortality rate ratio or hazard ratio, comparing HIV-D infected to HIV-2 infected individuals. Define whether the MRR or HR is given.
54	Mortality rate ratio-12 adjusted	Adjusted mortality rate ratio or hazard ratio, comparing HIV-1 infected to HIV-2 infected individuals. Define whether the MRR or HR is given.
55	Mortality rate ratio-adj vars	List the variables for which the adjusted MRR, or HR was adjusted

FU = follow-up; CI = confidence interval; IQR = interquartile range; ART = antiretroviral therapy; PY = person-years of observation; MRR = mortality rate ratio; HR = hazard ratio

Table S.2. Quality assessment checklist

External validity			
1	Representative	+	Random sample general population
		±	Random sample subpopulation (e.g. all TB patients)
		-	Non-random sample
		x	No information provided in the paper
2	Participation rate	+	Participation >80%
		±	Participation 50-80%
		-	Participation <50%
		x	No information provided in the paper
Internal validity			
3	HIV testing quality	+	ELISA, followed by confirmation tests of which at least was PCR
		±	ELISA, followed by confirmation tests other than PCR (e.g. western blot, synthetic-peptide-based line immunoassay)
		-	ELISA only
		x	No information provided in the paper
Confounding factors			
4	Age	+	Given per HIV group
		-	Only overall estimate reported
		x	No information provided in the paper
5	Gender	+	Given per HIV group
		-	Only overall estimate reported

		x	No information provided in the paper
6	CD4+ count	+	Given per HIV group
		-	Only overall estimate reported
		x	No information provided in the paper
7	Viral load	+	Given per HIV group and per HIV-type
		-	Only overall estimate reported
		x	No information provided in the paper
8	Lost to FU	+	If the percentage participants in the final analysis was 80% or more of the initially included population, or if a full description of those lost to FU was not suggestive of bias
		-	If the percentage was less or if there was no good description given of the loss to FU
		x	No information provided in the paper
9	How handled 'no show' at clinic	+	If a participant did not show at the clinic visit a home visit was done, or only home visits were done, or the vital status was checked from registries
		-	Lost to FU if no-show
		x	No information provided in the paper
10	How were cases that were lost to follow-up handled?	+	PY of FU censored at time of lost to FU of lost cases
		-	Total PY of FU excluded of lost cases
		x	No information provided in the paper
11	ART use	+	No ART use during study period
		-	ART use during study period
		x	No information provided in the paper

TB = tuberculosis; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; FU = follow-up; PY = person-years; ART = antiretroviral therapy

REFERENCES

1. Schim van der Loeff MF, Awasana AA, Sarge-Njie R, van der Sande M, Jaye A, Sabally S, *et al.* Sixteen years of HIV surveillance in a West African research clinic reveals divergent epidemic trends of HIV-1 and HIV-2. *Int J Epidemiol* 2006; **35(5)**:1322-1328.

2. Hamel DJ, Sankale JL, Eisen G, Meloni ST, Mullins C, Gueye-Ndiaye A, *et al.* Twenty years of prospective molecular epidemiology in Senegal: changes in HIV diversity. *AIDS Res Hum Retroviruses* 2007; **23(10)**:1189-1196.

3. Whittle H, Morris J, Todd J, Corrah T, Sabally S, Bangali J, *et al.* HIV-2-infected patients survive longer than HlV-1-infected patients. *AIDS* 1994; **8**:1617-1620.

4. Poulsen AG, Aaby P, Larsen O, Jensen H, Naucler A, Lisse IM, *et al.* 9-year HIV-2-associated mortality in an urban community in Bissau, west Africa. *Lancet* 1997; **349(9056)**:911-914.

5. Esbjörnsson J, Mansson F, Kvist A, Isberg PE, Nowroozalizadeh S, Biague AJ, *et al.* Inhibition of HIV-1 disease progression by contemporaneous HIV-2 infection. *N Engl J Med* 2012; **367(3)**:224-232.

6. Al-Harthi L, Owais M, Arya SK. Molecular inhibition of HIV type 1 by HIV type 2: effectiveness in peripheral blood mononuclear cells. *AIDS Res Hum Retroviruses* 1998; **14(1)**:59-64.

7. Arya SK, Gallo RC. Human immunodeficiency virus (HIV) type 2-mediated inhibition of HIV type 1: a new approach to gene therapy of HIV-infection. *Proc Natl Acad Sci U S A* 1996; **93(9)**:4486-4491.

8. Kokkotou EG, Sankale JL, Mani I, Gueye-Ndiaye A, Schwartz D, Essex ME, *et al.* In vitro correlates of HIV-2-mediated HIV-1 protection. *Proc Natl Acad Sci U S A* 2000; **97(12)**:6797-6802.

9. Rappaport J, Arya SK, Richardson MW, Baier-Bitterlich G, Klotman PE. Inhibition of HIV-1 expression by HIV-2. *J Mol Med (Berl)* 1995; **73(12)**:583-589.

10. Alabi AS, Jaffar S, Ariyoshi K, Blanchard T, Schim van der Loeff MF, Awasana AA, *et al.* Plasma viral load, CD4 cell percentage, HLA and survival of HIV-1, HIV-2, and dually infected Gambian patients. *AIDS* 2003; **17(10)**:1513-1520.

11. Nkengasong JN, Kestens L, Ghys PD, Koblavi-Deme S, Otten RA, Bile C, *et al.* Dual infection with human immunodeficiency virus type 1 and type 2: impact on HIV type 1 viral load and immune activation markers in HIV-seropositive female sex workers in Abidjan, Ivory Coast. *AIDS Res Hum Retroviruses* 2000; **16(14)**:1371-1378.

12. Schim van der Loeff MF, Jaffar S, Aveika AA, Sabally S, Corrah T, Harding E, *et al.* Mortality of HIV-1, HIV-2 and HIV-1/HIV-2 dually infected patients in a clinic-based cohort in The Gambia. *AIDS* 2002; **16(13)**:1775-1783.

13. van Tienen C, Schim van der Loeff M, Peterson I, Cotten M, Andersson S, Holmgren B, *et al.* HTLV-1 and HIV-2 infection are associated with increased mortality in a rural West African community. *PLoS One* 2011; **6(12)**:e29026.

14. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008; **61(4)**:344-349.

15. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, *et al.* The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses. In: *3rd Symposium on Systematic Reviews: beyond the basics. Improving quality and impact.*; 2000.

16. Martinez-Steele E, Awasana AA, Corrah T, Sabally S, van der Sande M, Jaye A, *et al.* Is HIV-2- induced AIDS different from HIV-1-associated AIDS? Data from a West African clinic. *AIDS* 2007; **21(3)**:317-324.

17. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21(11)**:1539-1558.

18. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315(7109)**:629-634.

19. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6(7)**:e1000097.