Supplementary Materials for

**HIV-1 Concentrations in Human Breast Milk Before and After Weaning**

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The PDF file includes:

- Fig. S1. Scatter plots showing associations between breast milk HIV-1 RNA concentrations in copies per milliliter, HIV-1 DNA concentration in copies per milliliter, and HIV-1 DNA concentration in copies per 1 million cell equivalents stratified by feeding practice at 4.5 months.
- Fig. S2. Flowchart of HIV-infected women who had breast milk HIV measurements.
- Breast-feeding and weaning questionnaires
Breastfeeding Practice  

![Graph showing associations between breast milk HIV-1 RNA concentrations and cellular virus in breast milk](image)

**Breastfeeding Practice**  
- O (---) Continued BF  
- + (---) Stopped BF

### Table: Spearman Coefficient and p-value

<table>
<thead>
<tr>
<th></th>
<th>Continued Breastfeeding</th>
<th>Stopped Breastfeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spearman Coefficient</strong></td>
<td><strong>Cellular virus in whole breast milk, log_{10} DNA copies/ml</strong></td>
<td><strong>Cellular virus, log_{10} DNA copies/10^6 cell equivalents</strong></td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>N=86</td>
<td>N=129</td>
</tr>
<tr>
<td>Cell-free virus in breast milk, log_{10} RNA copies/ml</td>
<td>0.49 &lt;0.0001</td>
<td>0.53 &lt;0.0001</td>
</tr>
<tr>
<td>Cellular virus in whole breast milk, log_{10} DNA copies/10^6 cell equivalents</td>
<td>0.80* &lt;0.0001</td>
<td>0.58* &lt;0.0001</td>
</tr>
</tbody>
</table>

*p<0.0001 for test of interaction with weaning

**Fig. S1.** Scatter plots showing associations between breast milk HIV-1 RNA concentrations in copies/ml, HIV-1 DNA concentration in copies/ml and HIV-1 DNA concentration in copies per 1 million cell equivalents (1M) stratified by feeding practice at 4.5 months.
Fig. S2. Flowchart of HIV-infected women who had breast milk HIV measurements
Clinic:  
☐ George  ☐ Chawama  

Date of visit:  


StudyID Child

Complete one form for every surviving child. If twins, complete 2 forms (one form for each child).

Indicate how many forms per mother  
☐ One form only  ☐ Two forms  

Form refers to:  
☐ Singleton  ☐ Twin 1  ☐ Twin 2  

Count exact number of weeks since last visit (if less than 1 week, write 00)

Closet to which scheduled visit:  
☐ 1 week  ☐ 1 month  ☐ 2 months  ☐ 3 months  ☐ 4 months  

Interviewer Initials:  

Respondent:  
☐ Mother  ☐ Other, who?_____________________

Is mother still primary carer of baby?  
☐ Yes  ☐ No  

IF NO, who takes care of baby?

☐ Father  

☐ Grandmother  

☐ Other family member  

☐ Other_____________________

**Part A: Feeding Practices**

**Time Interval 1:**

| 1 day | Last 6 days | Previous 3 weeks since last clinic visit |

I'm going to ask you about all the foods and liquids your baby has received in the last day. This includes foods and liquids that you gave your baby, and food and liquid that you know has been given to your baby by other people. Please answer truthfully. We need to know everything that the baby received.

1. Since yesterday morning until now, has your baby been fed directly from the breast?

☐ Yes  ☐ No  ☐ Don't know

2. Yesterday during the day, how many times did you breast-feed (put baby to the breast)?

Write 00 if none  

Number of times.

3. Last night (between sunset and sun up today), how many times did you breast-feed (put baby to the breast)?

Number of times.

4. Since yesterday, has your baby been fed any expressed breast milk?

☐ Yes  ☐ No  ☐ Don't know

Write 00 if none  

Number of feeds.

5. Yesterday during the day, how many feeds of expressed breast milk has your baby received?

Number of feeds.

6. Last night, how many feeds of expressed breast milk has your baby received?
7. Since yesterday, has your baby been given any plain water to drink?  

8. Since yesterday, has your baby been given any non-milk liquids to drink other than plain water? (By non-milk liquids we mean sugar water, tea with or without milk and sugar, juice, coke fanta, other cooldrinks, gripe water).  

9. Since yesterday, has your baby been given any non-human milks to drink? (This includes infant formula, animal milks, powdered milk)  

10. Since yesterday, has your baby been given any semi-solid or solid food? (This includes thin or thick porridge, commercial baby foods, vegetable/meat or other family foods)  

11. Since yesterday, has your baby been given any fermented food products (such as fermented milks, sour milk, fermented cereals, munkoyo, yogurt)?  

12. Since yesterday, has your baby been given any medicines or vitamins by mouth?  

13. Since yesterday, has your baby been given anything else by mouth not already mentioned?  

What was it?____________________________________________________________  

Time Interval 2:  

<table>
<thead>
<tr>
<th>1 day</th>
<th>Last 6 days</th>
<th>Previous 3 weeks since last clinic visit</th>
</tr>
</thead>
</table>

You have now told me about yesterday. Now I’d like you to think about all the time between yesterday back to one week ago.  
(Note to interviewer, e.g. If today is Monday, we are interested in the time between this hour on Sunday back until last Monday this hour). Please think back carefully and report everything the baby consumed. 

14. On how many days within the last week did baby consume..  

<table>
<thead>
<tr>
<th>Not given</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Everyday</th>
<th>Don't know</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Breast milk from the breast during the day</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2. Breast milk from the breast during the night</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>3. Expressed breast milk during the day</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>4. Expressed breast milk during the night</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>5. Plain water</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>6. Non-milk liquid</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>7. Non-human milk</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>8. Semi-solids or solids</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>9. Fermented milk or fermented cereal</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>10. Medicine</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>11. Anything else</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
**Time Interval 3:**

<table>
<thead>
<tr>
<th>Time Interval 3:</th>
<th>1 day</th>
<th>Last 6 days</th>
<th>Previous 3 weeks since last clinic visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Breast milk from the breast during the day</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2. Breast milk from the breast during the night</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>3. Expressed breast milk during the day</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>4. Expressed breast milk during the night</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>5. Plain water</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>6. Non-milk liquid</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>7. Non-human milk</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>8. Semi-solids or solids</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>9. Fermented milk or fermented cereal</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>10. Medicines</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>11. Anything else</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

**Time Interval 4:**

<table>
<thead>
<tr>
<th>All the time since last clinic visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>16. If anything other than breast milk was given, what was the youngest age of the child when it was first given?</td>
</tr>
<tr>
<td>a. Plain water</td>
</tr>
<tr>
<td>b. Non-milk liquids (not milk)</td>
</tr>
<tr>
<td>c. Non-human milks (formula, cows milk)</td>
</tr>
<tr>
<td>d. Semi-solids or solids</td>
</tr>
<tr>
<td>e. Fermented milk or fermented cereals</td>
</tr>
<tr>
<td>f. Anything else, what</td>
</tr>
</tbody>
</table>

17. Has the baby consumed anything from a bottle with a plastic teat? ☐ Yes ☐ No

18. Since the last clinic visit, have you expressed any breast-milk either for the baby to drink or for other reasons? ☐ Yes ☐ No
19. Why did you express breast-milk? Record the main reason.

☐ Did not express milk
☐ Following the advice of the study counsellors for group A.
☐ To relieve breast pain/engorgement
☐ Had to be separated from baby
☐ For heat-treatment before feeding
☐ Other-reasons ____________

20. Why were liquids or solids other than breast-milk given to baby? Record the one main reason.

☐ Not given
☐ infant wanted other liquids or solids
☐ insufficient milk
☐ advised to give by husband, family member or others
☐ mother not feeling well enough to breast-feed
☐ baby sick or crying
☐ mother was away and baby was cared for by someone else
☐ Other-reasons ________________________

21. Has anyone else (beside mother) ever breast-fed this infant (put baby to breast/wet-nurse)?

☐ Yes ☐ No ☐ Don't know

22. On how many days did wet-nursing occur? (write 00 if never, write 99 if don't know) ☐ ☐ Number of days

23. Since last visit, how many times in an average week did you go out of the house without your baby?

☐ None ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ Almost everyday

How long were you usually out of the house without your baby at any one time?

☐ Never ☐ Less than 1 hour ☐ 1-3 hours ☐ More than 3 hours

24. In your opinion, is it likely that others may have given baby other foods or liquids when you were not around?

☐ Yes ☐ No ☐ Don't know

25. Have you stopped all breast-feeding completely?

☐ Yes, stopped ☐ No, still breast-feeding ☐ Don't know

26. How old was your baby when you stopped breast-feeding him or her completely?

☐ Still breast feeding ☐ If stopped, exact age: ☐ ☐ months ☐ ☐ weeks ☐ ☐ days

27. Why did you stop breast-feeding your infant? Tick all that apply.

☐ Still breastfeeding ☐ Mother too sick to breast-feed
☐ Fear of transmitting HIV ☐ Infant too sick to breast-feed
☐ Advised to stop by health provider ☐ Infant no longer wanted to breast-feed
☐ Advised to stop by husband or other person ☐ Other-reasons ____________________________
☐ Separation from infant due to work ☐ Separation from infant due to hospitalization of mother or infant
Part B: Physical examination of baby and history 4:

1. Weight: [ ] [ ] kg
2. Length: [ ] [ ] cm

Examine baby’s skin:
3. Is there any evidence of a generalized rash? □ Yes □ No
   3a. If rash seen: □ Mild □ Moderate □ Severe

Look in baby’s mouth:
4. Are there any sores, lesions or ulcers in baby’s mouth? □ Yes □ No
5. Are there any white patches in baby’s mouth or throat consistent with thrush/candida? □ Yes □ No
6. Can teeth be seen? □ Yes □ No If teeth seen, count how many? [ ]

7. Check child clinic card. Is baby up to date with his or her vaccinations? If one needs to be given today, please ensure that the child gets it. □ Yes □ No □ Clinic card unavailable

8. Since last visit, have you taken your baby to any health care provider because he or she was sick (health care providers include going to the clinic or hospital, private doctor or GP, a chemist or pharmacist, a traditional healer)? □ Yes □ No

9. Since the last visit, have you brought your baby to a clinic or hospital because he/she was sick? □ Yes □ No
   If yes, record the reasons from clinic chart: CHECK ALL THAT APPLY
   □ pneumonia/breathing difficulty/cough □ failure to thrive
   □ meningitis □ malaria/fever
   □ diarrhea □ Other __________________________

10. Since last visit, has your baby been admitted to hospital or had to spend one or more night in hospital? □ Yes □ No (IF YES, NEEDS HOSPITALIZATION FORM)

11. Have you given your baby any medicine or treatment since last clinic visit?
   □ No medicine
   □ Oral rehydration solution ORS/sugar-salt solution
   □ Medicine from the clinic or hospital What? __________________________
   □ Medicine bought at the chemist or shop with no prescription.
   □ Medicine from the traditional healer
   □ Enema
   □ Other, what? __________________________

12. Since last clinic visit, has your baby had any episodes of diarrhoea, by which we mean 3 or more loose or watery stools per day for more than 2 consecutive days.
   □ Yes □ No □ Don't know
   If any episodes, how many episodes since the last clinic visit? [ ] [ ]
   For how many days did the longest episode last? [ ] [ ]
Part C: Physical examination of mother

1. Weight: ___________ kg

2. Since last visit, have you had to go to a clinic or hospital because you were sick?  
   □ Yes  □ No

3. Since last visit, have you been admitted to hospital?  
   □ Yes  □ No  
   IF YES, NEEDS HOSPITALIZATION FORM

4. Since the last clinic visit, have you had a menstrual period?  
   □ Yes  □ No

5. Are you using any method of family planning?  
   □ Yes  □ No  
   IF YES, which one:
   □ Condoms  □ Pills  □ Injectable  □ IUD  □ Other, what? ________________

Examine breasts

1. Is there evidence of engorgement (generalized swelling of the breast due to milk or fluid accumulation resulting in enlarged and swollen breasts)?
   □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

2. Does any area of the breast appear red or shiny?
   □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

3. Are the nipples sore or swollen?
   □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

4. Is there evidence of cracked or bleeding nipples?
   □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

5. Is there evidence of a blocked duct?
   □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

6. Is there evidence of a breast abscess?
   □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

7. Are there any whit patches on breasts, nipples or areola (candida symptoms)?
   □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

8. Are there any other problems with the breast, areola or nipple?
   □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

9. Ask mother: Do your breasts feel painful?
   □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

10. Ask mother: Have you had any breast problems since your visit?
    □ Yes, right breast only  □ Yes, left breast only  □ Yes, both breasts  □ No

11. IF any breast abnormalities, take mother's temperature: ___________ degrees C.

12. Ask mother: Have you had a fever since your last visit?  
    □ Yes  □ No
### Part D: Specimen collection

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Yes</th>
<th>No, explain</th>
<th>Not Needed</th>
<th>Needed, but couldn't...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heel-stick</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child blood sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother blood sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast milk samples:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Breast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Breast</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>