

## **SUPPLEMENTAL MATERIALS**

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**Supplemental Table 1. Participant and maternal baseline characteristics, caregiver-reported adherence measures, and correlates of INH adherence as indicated by a positive urine isoniazid result\***

Characteristic	Median (IQR) or n (%) N=97	RR (95% CI)	p
<b>Infant Characteristics</b>			
Infant age (weeks)	6.3 (6.0-6.4)	1.0 (1.0-1.0) <sup>a</sup>	0.17
Weight-for-age Z (WAZ) score (at visit)	0.2 (-0.6-0.8)	1.3 (1.1-1.6)	0.002
Male	56 (57.7)	1.0 (0.7-1.5)	0.84
Received ARVs for PMTCT	95 (97.9)	0.5 (0.1-2.1)	0.34
Study visit	--	0.9 (0.8-1.0)	0.17
Time since enrollment (months)	2.1 (1.2-4.7)	1.0 (0.9-1.0)	0.16
<b>Maternal Characteristics on enrollment</b>			
Maternal age (years)	26 (24-30)	1.0 (1.0-1.1) <sup>b</sup>	0.51
Secondary education started	43 (44.3)	1.5 (1.1-2.2)	0.02
Maternal CD4 (cells/mm <sup>3</sup> ) <sup>c</sup> (n=83)	460 (313-646)	1.0 (1.0-1.0)	0.34
Maternal HIV RNA (copies/ml) <sup>c</sup> (n=89)	0 (0-0)	1.0 (1.0-1.0)	0.80
HIV viral load undetectable <sup>d</sup>	68 (76.4)	1.1 (0.7-1.6)	0.68
HIV viral load <1000 (copies/ml)	83 (93.3)	2.1 (1.1-4.0)	0.02
Maternal ART current use	97 (100)	--	--
Maternal ART initiation timing			
Before pregnancy	69 (71.1)	0.9 (0.6-1.4)	0.62
During/after pregnancy <sup>e</sup>	28 (28.9)	ref	
Maternal history of TB	10 (10.3)	0.8 (0.4-1.3)	0.35
Maternal IPT any use	73 (75.3)	1.3 (0.8-2.1)	0.33
Maternal IPT current use	26 (37.0)	0.7 (0.5-1.1)	0.11
Maternal IPT initiation timing			
Before pregnancy	20 (27.4)	0.9 (0.6-1.5)	0.72
During pregnancy	44 (60.3)	0.7 (0.5-1.1)	0.12
After pregnancy	9 (12.3)	ref	
<b>Residential Characteristics on enrollment</b>			
Persons in household	4 (4-4)	1.0 (0.8-1.2)	0.93
Rooms in household	2 (1-3)	1.1 (1.0-1.2)	0.13
Flush toilet in home	11 (11.3)	1.4 (0.9-2.2)	0.10
Electricity in home	65 (67.0)	1.1 (0.7-1.6)	0.79
Running water in home	20 (20.6)	1.1 (0.7-1.9)	0.70
<b>Caregiver-reported adherence at visit</b>			
	<b>N=155</b>		
Median time since last INH dose (hours)	15.1 (5.0-17.1)	1.0 (1.0-1.0)	0.20
Last INH dose (hours)			
<24	134 (86.5)	4.9 (0.8-28.9)	0.08
24-48	14 (9.0)	4.3 (0.7-25.4)	0.10
>48	7 (4.5)	ref	
Optimal INH use throughout the study <sup>f</sup>	93 (60.0)	0.9 (0.6-1.4)	0.78
Optimal INH use since last study visit <sup>f</sup>	149 (96.1)	3.7 (0.7-20.1)	0.13
No missed doses in past 3 days	136 (87.7)	2.4 (1.0-5.6)	0.05

<sup>a</sup> Age at study visit (per each additional week)

<sup>b</sup> Age (per each additional year)

<sup>c</sup> CD4 and VL data collected from routine programmatic data

<sup>d</sup> HIV viral load < 20 copies/ml

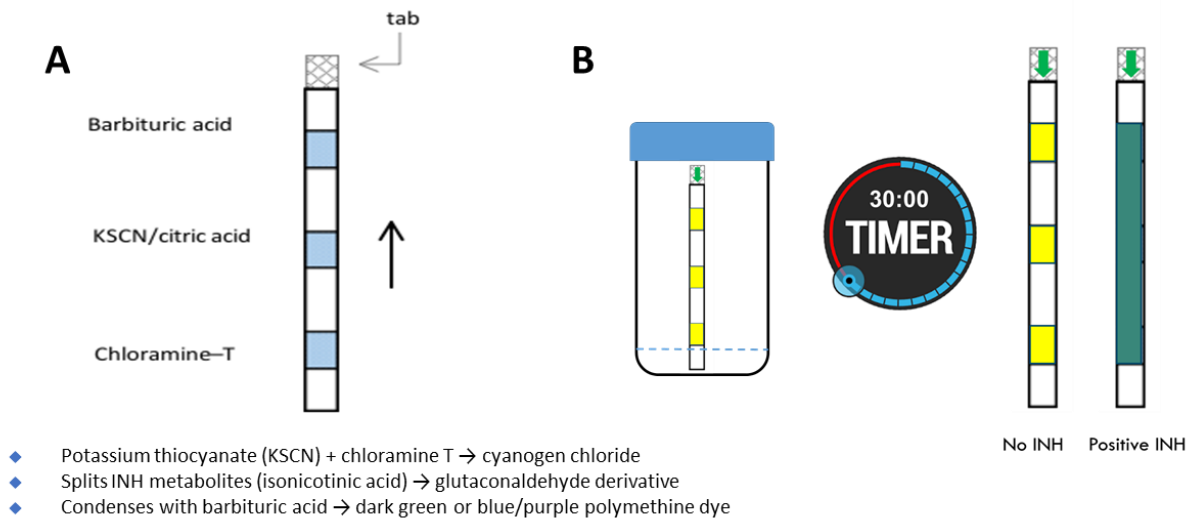
<sup>e</sup> Includes 1 mother who initiated ART after delivery but before study enrollment

<sup>f</sup> ≥ 90% reported adherence

\*Infant, maternal, and residential characteristics are from baseline with exception of weight-for-age (WAZ) score, study visit, and time since enrollment which is from the visit that urine testing occurred. Caregiver-reported adherence is from the visit that urine testing occurred.

## Development of an in-house urine INH metabolite test

To detect urine INH metabolites in urine, a modified Arkansas method was used to detect a color change produced when the INH metabolite isonicotinic acid reacts with cyanogen chloride and barbituric acid impregnated into the dipstick (**Supplemental Figure**). The INH metabolite is typically detectable in urine within 24-30 hours of ingestion.



**Supplemental Figure. Development of a urine dipstick assay to detect INH metabolites as a biomarker-based adherence measure.** *Panel A:* Development of urine dipstick assay using a modified Arkansas method to detect the INH metabolite isonicotinic acid. By reversing the order of reagents, a lower concentration of reagents was required. *Panel B:* The INH dipstick is placed so that the bottom edge of the dipstick is in contact with the urine, allowing the urine to wick up the dipstick and then reviewed for color change at 30 minutes. A positive test is defined as a color change of green or blue/purple.

Deposition of chemical reagents was facilitated by the dispense platform equipped with FrontLine dispensers (BioDot ZX1010). Cotton fiber membranes (Ahlstrom In Vitro Diagnostic Cotton Fiber Grade 222) were cut into 60 mm x 200 mm strips and fixed onto PVC backing cards (Diagnostic Consulting Network MIBA-020). The reagents, barbituric acid (1.5% w/v (g/mL), pH 5.2, Sigma Aldrich 185698); chloramine-T trihydrate (15% w/v, Sigma Aldrich 402869); and a solution of potassium thiocyanate (60% w/v, Sigma Aldrich P3011) with citric acid monohydrate (8% w/v, Sigma Aldrich C1909); were solubilized in filtered deionized water. Then using the striper, reagents were dispensed onto the assembled cards until each 4 mm wide section of the card contained the following amount of each reagent as shown in **Supplemental Figure, Panel A**; barbituric acid: 1.80 mg; chloramine-T trihydrate: 2.52 mg; potassium thiocyanate: 1.08 mg. It should be noted that in order to prevent the reagents from blending together, the cards were dried for 5 minutes in an oven at 55 °C. Once the cards were completed and dried, they were stored in a desiccator box until they were ready to be cut into strips. The final 4 mm wide strips were cut using a guillotine strip cutter (Kinbio ZQ2002).

To verify the performance of these strips, we evaluated these strips using mock INH metabolite solutions of isonicotinic acid (Sigma Aldrich I17508) at 1, 5, 10, 25, 50, and 100 µg/mL. 0.5 mL of each sample was added to its respective culture tube (10 mm x 75 mm) before a test strip was placed in the tube. The target limit of detection was 5 µg/mL, was chosen to match the limit of detection reported by Becton Dickinson for their discontinued commercial INH detection strip BD-Taxo™ - INH Test Strips (10-231743). The strips that performed as expected were stored in sealed mylar bags with desiccant packets until used.

**Supplemental Table 2. INH dipstick assay results overall and by visit**

<b>INH dipstick color change</b>	<b>Total N=155 n (%)</b>	<b>Visit 2 10 weeks of age N=48 n (%)</b>	<b>Visit 3 3 months of age N=49 n (%)</b>	<b>Visit 4 6 months of age N=25 n (%)</b>	<b>Visit 5 9 months of age N=14 n (%)</b>	<b>Visit 6 12 months of age N=9 n (%)</b>	<b>Visit 7 12 months post enrollment N=10 n (%)</b>
Yes	77 (49.7)	29 (60.4)	26 (53.1)	12 (48.0)	1 (7.1)	3 (33.3)	6 (60.0)
No	78 (50.3)	19 (39.6)	23 (46.9)	13 (52.0)	13 (92.9)	6 (66.7)	4 (40.0)

**Supplemental Table 3. INH dipstick result and caregiver-reported adherence measures overall and by visit**

Caregiver-reported timing of last INH use (hours)																					
INH dipstick color change	Total N=155 n (%)			Visit 2 10 weeks of age N=48 n (%)			Visit 3 3 months of age N=49 n (%)			Visit 4 6 months of age N=25 n (%)			Visit 5 9 months of age N=14 n (%)			Visit 6 12 months of age N=9 n (%)			Visit 7 12 months post enrollment N=10 n (%)		
	<24 n=134	24-48 n=14	>48 n=7	<24 n=44	24-48 n=3	>48 n=1	<24 n=42	24-48 n=4	>48 n=3	<24 n=22	24-48 n=3	>48 n=0	<24 n=9	24-48 n=3	>48 n=2	<24 n=8	24-48 n=0	>48 n=1	<24 n=9	24-48 n=1	>48 n=0
Yes	69 (51.5)	7 (50.0)	1 (14.3)	26 (59.1)	3 (100)	0 (0)	24 (57.1)	1 (25.0)	1 (33.3)	10 (42.5)	2 (66.7)	0 (0)	1 (11.1)	0 (0)	0 (0)	3 (37.5)	0 (0)	0 (0)	5 (55.6)	1 (100)	0 (0)
No	65 (48.5)	7 (50.0)	6 (85.7)	18 (40.9)	0 (0)	1 (100)	18 (42.8)	3 (75.0)	2 (66.7)	12 (54.6)	1 (33.3)	0 (0)	8 (88.9)	3 (100)	12 (100)	5 (62.5)	0 (0)	1 (100)	4 (44.4)	0 (0)	0 (0)
Caregiver-reported missed dose in past 3 days																					
INH dipstick color change	Total N=155 n (%)		Visit 2 10 weeks of age N=48 n (%)		Visit 3 3 months of age N=49 n (%)		Visit 4 6 months of age N=25 n (%)		Visit 5 9 months of age N=14 n (%)		Visit 6 12 months of age N=9 n (%)		Visit 7 12 months post enrollment N=10 n (%)								
	No n=136	Yes n=19	No n=46	Yes n=2	No n=46	Yes n=3	No n=21	Yes n=4	No n=5	Yes n=9	No n=8	Yes n=1	No n=	Yes n=0							
Yes	72 (52.9)	5 (26.3)	28 (60.8)	1 (50.0)	25 (54.4)	1 (33.3)	9 (42.9)	3 (75.0)	1 (20.0)	0 (0)	3 (37.5)	0 (0)	6 (60.0)	0 (0)							
No	64 (47.1)	14 (73.7)	18 (39.1)	1 (50.0)	21 (45.7)	2 (66.7)	12 (57.1)	1 (25.0)	4 (80.0)	9 (100)	5 (62.5)	1 (100)	4 (40.0)	0 (0)							
Caregiver-reported optimal INH use since last visit *																					
INH dipstick color change	Total N=155 n (%)		Visit 2 10 weeks of age N=48 n (%)		Visit 3 3 months of age N=49 n (%)		Visit 4 6 months of age N=25 n (%)		Visit 5 9 months of age N=14 n (%)		Visit 6 12 months of age N=9 n (%)		Visit 7 12 months post enrollment N=10 n (%)								
	Optimal n=149	Suboptimal n=6	Optimal n=47	Suboptimal n=1	Optimal n=46	Suboptimal n=3	Optimal n=25	Suboptimal n=0	Optimal n=13	Suboptimal n=1	Optimal n=8	Suboptimal n=1	Optimal n=10	Suboptimal n=0							
Yes	76 (51.0)	1 (16.7)	29 (61.7)	0 (0)	25 (54.4)	1 (33.3)	12 (48.0)	0 (0)	1 (7.7)	0 (0)	3 (37.5)	0 (0)	6 (60.0)	0 (0)							
No	73 (49.0)	5 (83.3)	18 (38.3)	1 (100)	21 (45.7)	2 (66.7)	13 (52.0)	0 (0)	12 (92.3)	1 (100)	5 (62.5)	1 (100)	4 (40.0)	0 (0)							
Caregiver-reported optimal INH use throughout study*																					
INH dipstick color change	Total N=155 n (%)		Visit 2 10 weeks of age N=48 n (%)		Visit 3 3 months of age N=49 n (%)		Visit 4 6 months of age N=25 n (%)		Visit 5 9 months of age N=14 n (%)		Visit 6 12 months of age N=9 n (%)		Visit 7 12 months post enrollment N=10 n (%)								
	Optimal n=93	Suboptimal n=62	Optimal n=30	Suboptimal n=18	Optimal n=27	Suboptimal n=22	Optimal n=17	Suboptimal n=8	Optimal n=8	Suboptimal n=6	Optimal n=5	Suboptimal n=4	Optimal n=6	Suboptimal n=4							
Yes	47 (50.5)	30 (48.4)	19 (63.3)	10 (55.6)	16 (59.3)	10 (45.5)	7 (41.2)	5 (62.5)	0 (0)	1 (16.7)	2 (40.0)	1 (25.0)	3 (50.0)	3 (75.0)							
No	46 (49.5)	32 (51.6)	11 (36.7)	8 (44.4)	11 (40.7)	12 (54.6)	10 (58.8)	3 (37.5)	8 (100)	5 (83.3)	3 (60.0)	3 (75.0)	3 (50.0)	1 (25.0)							

\*≥ 90% reported adherence

**Supplemental Table 4. INH dipstick assay compared to commercial test**

		IsoScreen N=15	
		Positive n (%)	Negative n (%)
<b>INH dipstick</b>			
Positive	n (%)	7 (87.5)	1 (14.3)
Negative	n (%)	1 (12.5)	6 (85.7)
Sensitivity %		87.5%	
Specificity %		85.7%	
Agreement %		86.7%	
Kappa (95% CI)		kappa 0.73, p=0.002	

**Supplemental Table 5. INH dipstick and IsoScreen result and caregiver-reported adherence measures**

	Total N=15 n (%)	Caregiver-reported timing of last INH use (hours) N=15 n (%)			Caregiver-reported missed dose in past 3 days N=15 n (%)		Caregiver-reported optimal INH use since last visit* N=15 n (%)	
		<24 n=13	24-48 n=2	>48 n=0	No n=13	Yes n=2	Optimal n=15	Suboptimal n=0
<b>Color change</b>								
Yes	7 (46.7)	6 (46.2)	1 (50.0)	--	7 (53.9)	0 (0.0)	7 (46.7)	--
No	8 (53.3)	7 (53.9)	1 (50.0)	--	6 (46.2)	2 (100.0)	8 (53.3)	--
<b>IsoScreen</b>								
Yes	8 (53.3)	7 (53.9)	1 (50.0)	--	8 (61.5)	0 (0.0)	8 (53.3)	--
No	7 (46.7)	6 (46.2)	1 (50.0)	--	5 (38.5)	2 (100.0)	7 (46.7)	--

\*≥ 90% reported adherence since last study visit