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TIMELINESS OF EARLY INFANT HIV DIAGNOSIS AND ASSOCIATED FACTORS AMONG HIV-EXPOSED INFANTS IN PUBLIC HOSPITALS, ARSI ZONE, SOUTHEAST ETHIOPIA, 2025

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List of Abbreviations and Acronyms

AIDS	Acquired Immune Deficiency Syndrome
AOR	Adjusted Odds Ratio
ART	Antiretroviral Therapy
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
COR	Crude Odds Ratio
DBS	Dried Blood Spot
DNA	Deoxyribonucleic Acid
EBF	Exclusive breastfeeding
EID	Early Infant Diagnosis
EMTCT	Elimination of Mother-to-Child Transmission
EPHI	Ethiopian Public Health Institute
HEI	HIV Exposed Infant
HIV	Human Immunodeficiency Virus
MTCT	Mother to Child Transmission
NAT	Nucleic acid testing
PCR	Polymerase Chain Reaction
PNP	postnatal prophylaxis
POC	point of care
SPSS	Statistical Package for the Social Sciences
UNAIDS	Joint United Nations Programme on HIV/AIDS
PMTCT	Prevention of Mother-to-Child Transmission
WHO	World Health Organization

ABSTRACT

Background: Early infant diagnosis (EID) is critical for reducing HIV-related morbidity and mortality among HIV-exposed infants. The World Health Organization recommends virological testing within 4–6 weeks after birth. However, many low-resource settings, including Ethiopia, continue to experience suboptimal performance in achieving the ≥95% timeliness target.

Objective: To assess the timeliness of early infant HIV diagnosis and identify associated factors among HIV-exposed infants at public hospitals in Arsi Zone, Southeast Ethiopia.

Methods: A facility-based cross-sectional study was conducted through retrospective chart reviews from August 30 to September 30, 2025. A consecutive sample of 414 mother-infant pairs was drawn from three randomly selected public hospitals. Data were collected using KoboToolbox and analyzed in SPSS version 27. Factors associated with early diagnosis were identified using bivariate and multivariable logistic regression, with results presented as adjusted odds ratios (AOR) and 95% confidence intervals (CI).

Results: Among the 414 Human Immunodeficiency Virus-exposed infants enrolled (300 from Asella Referral and teaching Hospital, 62 from Robe Didea Hospital, and 52 from Abomsa Hospital), only 61.6% (255/414) had their first PCR sample collected within the recommended ≤45 days after birth. In multivariable analysis, reduced odds of timely EID were significantly associated with: maternal age <25 years (AOR = 0.043, 95% CI: 0.015–0.121) and >35 years (AOR = 0.257, 95% CI: 0.103–0.641); single (AOR = 0.119, 95% CI: 0.028–0.502) and divorced (AOR = 0.103, 95% CI: 0.025–0.427) marital status; rural residence (AOR = 0.124, 95% CI: 0.056–0.276); no antenatal care follow-up (AOR = 0.163, 95% CI: 0.076–0.349); home delivery (AOR = 0.083, 95% CI: 0.033–0.211); and mixed breastfeeding (AOR = 0.126, 95% CI: 0.049–0.321).

Conclusion and recommendations:

Early infant diagnosis timeliness in the Arsi Zone remains substantially below the global target. Interventions should prioritize young and older mothers, unmarried women, rural residents, and those with home deliveries or inadequate antenatal care to improve early infant diagnosis coverage.

Keywords: Early infant diagnosis, HIV-exposed infants, polymerase chain reaction, timeliness, Ethiopia, Arsi Zone

1. Background

1.1 . Introduction

Human immunodeficiency virus (HIV) infection in infants represents a distinct and persistent public health challenge, characterized by vertical transmission dynamics and accelerated disease progression without intervention(1).Pediatric AIDS cases were first reported approximately 18 months after the initial adult cases in the early 1980s (2).Globally, an estimated 2.4 million children and adolescents were living with HIV in 2023, with approximately 712 new pediatric infections and 250 AIDS-related deaths occurring daily (3). Concurrently, 1.3 million new HIV infections and 630,000 AIDS-related deaths were recorded worldwide in the same year (4).Sub-Saharan Africa bears the greatest burden, accounting for approximately two-thirds of all people living with HIV globally(5).Among regions affected, Sub-Saharan Africa is the most severely affected area globally, accounting for over two-thirds of all individuals living with HIV(6).A major route of HIV infection in infants is mother-to-child transmission, accounting for more than 90% of infections that occur during pregnancy, childbirth, or breastfeeding(7). PMTCT strategies effectively reduce transmission risk when properly implemented(8). Early infant diagnosis (EID) through DNA polymerase chain reaction (PCR) testing at 4–6 weeks of age enables timely ART initiation for infected infants (9). The World Health Organization recommends virological testing within this window, with immediate treatment for positive cases(10).The cornerstone of successful PMTCT is Early Infant Diagnosis (EID). According to the WHO, EID involves virological testing using DNA PCR at 4–6 weeks of age (11). And Positive results trigger immediate ART initiation (within 7 days), confirmatory testing, and close follow-up; negative results require continued monitoring until final antibody testing 12–18 months after cessation of breastfeeding (for at least 6 weeks) (12). Many countries in sub-Saharan Africa (SSA) are facing significant challenges in achieving the 95% target for early infant diagnosis (EID) of HIV services to end this infection by 2030 in children. It's crucial to recognize the impact this has on families and communities, and efforts must be intensified to support better outcomes for our most vulnerable infants (13).

1.2. STATEMENT OF THE PROBLEM

Globally, HIV remains a major public health challenge, with 1.4 million children living with the virus and 120,000 new infections alongside 76,000 AIDS-related deaths recorded in 2023 (14). Limited uptake of infant HIV testing and persistent gaps in prevention of mother-to-child transmission (PMTCT) programs continue to drive this burden, contributing to high mortality among HIV-exposed infants (15). In the Americas, delayed maternal diagnosis and inadequate prenatal care have increased missed opportunities for early infant HIV diagnosis, with undiagnosed infants facing a 2.3-fold higher risk of mortality (16). Across Asia, only 37% of HIV-exposed infants receive early infant HIV diagnosis within two months, MTCT rates reach 23%, and delays in early infant HIV diagnosis are linked to an odds ratio of 2.1 for progression to AIDS (17).

In Sub-Saharan Africa, where the burden is greatest, uptake of early infant HIV diagnosis remains below global targets. In Ethiopia, a systematic review and meta-analysis of national data found that only 64.8% of HIV-exposed infants received early infant HIV diagnosis within 6 weeks, far below the national target of ≥95% (18). Ending pediatric HIV requires meeting the global 95–95–95 targets and ensuring elimination of mother-to-child transmission (EMTCT) by 2030 (19). Central to these goals is timely early infant HIV diagnosis, as delays in diagnosis significantly worsen survival outcomes for HIV-exposed infants (20).

WHO recommends Early Infant Diagnosis (EID) of HIV at 4–6 weeks of age to enable prompt ART initiation. Early detection, immediate treatment, and prophylactic antibiotics against opportunistic infections significantly reduce morbidity and mortality, greatly improving survival among HIV-infected children (15, 21). Since 2012, Option B+ lifelong ART for all pregnant and breastfeeding women has been the global PMTCT standard (21). Ethiopia initially implemented Option A in 2011 (22). Ethiopia transitioned to Option B+ in August 2012, expanding it nationwide (23).

Despite these policy advances, substantial gaps remain in achieving timely EID.for example, Global estimates show that only 63% of HIV-exposed infants received EID at the recommended 4–6 weeks in 2020 (24), in Thailand's 65% coverage (25). In Africa, EID coverage ranges from 39% to 72%. In Ethiopia, a national systematic review confirmed that only 64.8% receive EID within 6 weeks (20). In different African countries (Uganda, Ghana, South Africa, Zambia, Tanzania, Cameroon), EID coverage ranges from 39% to 72% (26, 27, 28, 29). Although Ethiopia has made progress toward EMTCT and has achieved the second and third 95% targets nationally (30). Delays in EID persist and continue to impede full EMTCT

attainment (31). Disparities remain most pronounced among HIV-exposed infants, who are less likely to meet the first 95% target than adults (32). The country's PMTCT policy history, from Option A in 2011 (22). to the full adoption of Option B+ in 2012 (33, 34).

Globally, initiatives such as the UNAIDS 95–95–95 targets and WHO's EMTCT agenda stress that at least 95% of HIV-exposed infants should undergo early infant diagnosis (EID) within 4–6 weeks (35, 36).

To accelerate progress toward pediatric HIV elimination, countries have expanded Prevention of Mother-to-Child Transmission (PMTCT) programs, introduced point-of-care technologies such as GeneXpert, and strengthened referral systems. Despite these efforts, many low-resource settings, including Ethiopia, continue to fall short of the ≥95% timeliness threshold for Early Infant HIV Diagnosis (EID). At the local level, PMTCT services are available in public hospitals of the Arsi Zone. However, no study has assessed whether this zone is on track to meet the global target. Critically, the timeliness of the first DNA PCR test, the earliest and most decisive step in the EID cascade, and the PMCT's success have not been evaluated as a distinct outcome. Ethiopia's national performance remains below target, with only 64.8% of HIV-exposed infants tested within six weeks. This falls short of the UNAIDS 95-95-95 targets and the WHO's Elimination of Mother-to-Child Transmission (EMTCT) agenda for 2030. Addressing timeliness is therefore urgent to prevent avoidable deaths and accelerate progress toward elimination.

By focusing on the earliest point in the EID cascade, the first PCR test in this study provides localised, actionable evidence for Ethiopia's national HIV program and regional health planners. Findings were informed strategies to strengthen antenatal care uptake, promote exclusive breastfeeding, and expand facility-based delivery, thereby improving PMTCT service performance and advancing Ethiopia's pediatric HIV elimination goals. The absence of localized evidence on the timeliness of the first DNA PCR test in Arsi Zone represents a critical gap that must be addressed to identify bottlenecks and design targeted interventions aligned with the 2030 elimination agenda.

1.3. SIGNIFICANCE OF THE STUDY

From a scientific perspective, this study may be an original contribution by providing the first localized evidence on the timeliness of the initial DNA PCR test among HIV-exposed infants in Arsi Zone, Ethiopia. Isolating the earliest and most critical step of the EID cascade, it identifies specific determinants of delay that have not been previously documented in this setting. The large sample size, multi-facility design, and focus on five data points to see the success of PMCT in the zone over the last five years ago and fill a notable gap in the Ethiopian literature on PMTCT service delivery. These findings serve as a valuable reference for future research on EID timeliness, retention in care, and long-term outcomes of HIV-exposed infants. Programmatically, the results offer immediate, actionable insights for strengthening PMTCT and pediatric HIV services. Facility-specific data on turnaround times, testing platforms, and missed opportunities enable hospital managers and PMTCT focal persons to redesign workflows, integrate sample collection at delivery and immunization points, and prioritise GeneXpert utilisation. The identified barriers to rural residence, home delivery, and inadequate counselling provide clear entry points for targeted interventions to improve linkage from antenatal care to early infant testing and treatment.

At the policy level, this study directly informs Ethiopia's national efforts to achieve the UNAIDS 95–95–95 targets and eliminate mother-to-child transmission of HIV by 2030. By demonstrating that only 61.6% of infants in Arsi Zone are tested within the recommended timeframe, far below the 95% target, the evidence underscores the urgency of revising guidelines, scaling point-of-care diagnostics, and addressing geographic inequities. National HIV programs can use these localized findings to refine resource allocation, update performance indicators, and monitor progress toward validating EMTCT.

Locally, the Arsi Zone Health Department and Oromia Regional Health Bureau are primary beneficiaries. The study equips zonal planners with precise data to design context-specific interventions, such as transport subsidies for rural mothers, community-based follow-up systems, and stigma-reduction campaigns. Hospital administrators gain evidence to justify investments in staff training and supply chain improvements. Ultimately, mothers, families, and HIV-exposed infants in Arsi Zone will benefit from faster diagnosis, earlier treatment, and reduced morbidity, advancing both community health outcomes and Ethiopia's ambitious 2030 pediatric HIV elimination goal.

2. Literature review

2.1 Burden of HIV in INFANTS

HIV is spread vertically from mother to newborn through a process known as mother-to-child transmission (MTCT), which accounts for more than 90% of HIV infections in infants(37). HIV/AIDS transmission from mother to child (MTCT) is one of the major problems of the epidemic, particularly in environments with limited resources (21). The PMTCT program includes Early Infant Diagnosis (EID) as part of its vital service package (15). EID programs generally consist of collecting dried blood spot samples at clinics and transporting them to central laboratories equipped with NAT capabilities; lack of transportation and complex communication systems lead to extended turnaround times and potential loss to follow-up before the initiation of ART (38). For children under 18 months old, this entails EID testing directly for HIV DNA using the Polymerase Chain Reaction (PCR) method, which yields a conclusive diagnosis (39). This allows for early clinical assessment, antiretroviral treatment, and antibiotic prophylaxis against opportunistic infections for the newborns (40, 41) . Anti-HIV antibodies produced by HIV-positive mothers can survive the placenta and remain in the blood of their unborn children for up to 18 months (42). When found in early infants, these antibodies typically indicate maternal HIV exposure rather than actual infant HIV infection (43). Despite these recommendations, delays in EID remain a challenge, contributing to high mortality among HIV-infected infants (29).

2.2. The Crucial Role of the Initial PCR Test in the EID Cascade in the Diagnosis of HIV-Exposed Infants (HEIs)

The first PCR test serves as a pivotal cornerstone in the meticulous cascade of Early Infant Diagnosis (EID) for infants exposed to HIV (44). This vital assessment holds immense significance in identifying the presence of the virus at the earliest stages, allowing for timely intervention and care (44). As the initial line of defence in safeguarding the health of HIV-exposed infants, the PCR test empowers healthcare providers to navigate the complexities of HIV transmission, ensuring that vulnerable little lives receive the attention and support they desperately need (45). By unlocking the potential for immediate treatment and monitoring, the test is not merely a medical procedure; it represents hope and the promise of a healthier future for those at risk.

The first PCR test serves as the cornerstone routine of the Early Infant Diagnosis (EID) cascade, establishing the baseline for all subsequent testing and clinical interventions, also known as EID alone (15). Subsequent PCR tests are inherently dependent on the timing and results of the initial test, as the second PCR relies on the first, and the third antibody test is again influenced by both prior results and the duration of breastfeeding. Thus, the first PCR test not only determines the infant's diagnostic trajectory but also directly impacts the timing of ART initiation without waiting for the second PCR, which is tested at 12 months and the antibody test at 18 months (46).

Delays or missed opportunities in performing the first PCR test within 45 days alone can disrupt the entire continuum of care, also known as delayed infant diagnosis (47). Late testing has been associated with postponed follow-up tests, delayed ART initiation, and higher infant morbidity and mortality (48). Focusing on the predictors and timeliness of the first PCR test is central to the Early Infant Diagnosis (EID) process, as it represents the primary and most critical stage of HIV testing for exposed infants (49). While the second PCR test and final antibody test are important, their timing and performance entirely depend on the completion and outcome of the first PCR test, which establishes the foundation for the entire EID cascade (50).

2.3. The first delay in the EID context among HIV-exposed infants

This study focuses on the first delay factors within the three Delays Model as they pertain to the initiation of diagnostic processes. The first delay is the time from birth to the decision to seek the first PCR testing (51). Or Delay one is the Mothers' delay in early return or not bringing the infants to health facilities for the offer test (52). The second delay refers to the time taken to send the sample for DBS-PCR due to issues with the courier and postage system. Reported reasons for this delay include: the minimum number of samples required for courier service, poor cooperation from postal offices, loss of samples during transport, and the necessity to resend samples on two occasions (52). So, this study focuses on the date of sample collection rather than waiting for the release of results. Delays occurring after sample collection reflect facility-related or laboratory delays (the second and third delay), not patient-related delays, such as the decision to seek care or the time taken to reach the facility (first delay) (52).

2.4. Global and Policy Context on the Timeliness of Early Infant HIV Diagnosis among HIV-Tested, HIV-Exposed Infants (HEI)

Achieving timely EID is critical for reaching the global goal of ending pediatric HIV infections and creating an HIV-free generation by 2030 (15). The WHO recommends EID for all HIV-exposed infants at 4–6 weeks of age to ensure early initiation of antiretroviral therapy and reduce infant mortality (53). For children under 18 months, virological testing using PCR (DNA or RNA) provides a definitive diagnosis, making the timing of the first PCR test a crucial public health indicator (54). Delays beyond 45 days risk late detection which could delay the initiation of antiretroviral therapy for HIV-positive infants, increasing the risk of morbidity and mortality and compromising the effectiveness of PMTCT programs (55). Addressing first delay factors will contribute to improving EID coverage and timely initiation of antiretroviral therapy, advancing Ethiopia toward the 95–95–95% HIV targets and the elimination of pediatric HIV by 2030 (56).

2.5 Proportion of Timeliness of Early Infant HIV Diagnosis among HIV Tested HIV Exposed Infants.

A retrospective cohort study conducted in Norway involving 60 infants exposed to HIV infection from 2010 to 2017 indicates that approximately 92% of these infants received early infant diagnosis (EID) within the 6-week timeframe (57). A review conducted from 2010 to 2020 in the United Kingdom in 2023 showed that the take-up of EID within 6 weeks was 90% (58). In the Netherlands, in a 2020 retrospective study conducted among 110 HIV exposed infants, the uptake of EID at 6 weeks was around 90% (59). In Belgium, a cross-sectional study among 70 HIV exposed infants found that 85 % of the proportion of EID was within 6 weeks (60). In Italy, one study conducted in 2020 among 120 HIV exposed infants reported that around 88% utilization of EID for HIV infection within the 6 weeks (61). A 2017 observational study in Myanmar found that the timeliness of early infant diagnosis (EID) before 8 weeks of age was just 28% (62). A mixed-methods study across 62 centers in 11 Indian states found 78% of HIV-exposed infants had at least one test, but only 50% received their first PCR by 8 weeks (63). A mixed-method study conducted in Nigeria in 2023 shows that the magnitude of EID within 6 weeks was only 40.9% (64). A July 2021 study in Ngombe, Tanzania, found 85% of HIV-exposed infants received 4–6 week PCR testing, showing good coverage but gaps in universal early testing (65, 66). A 2017 to 2018 Kenyan pilot in four hospitals enrolled 624 HIV-exposed pairs; 76.6% completed birth PCR on time, 92.1% were tested within six weeks, and 66.8% completed both birth and six-week tests (67).

A 2016–2018 Kenyan study of 4,011 HIV-exposed infants found 66.5% were diagnosed late, with a median first test age of 3 months (68). A 2016–2017 study at Kisenyi Health Centre IV, Uganda, found that among 246 HIV-positive mother–infant pairs, only 21.9% of HIV-exposed infants were tested within 6–8 weeks, 53.7% were never tested, and 24.4% were tested late (69). In 2019, 75,000 infants in Uganda received their first HIV DNA PCR test, with only 71% tested within 2 months of birth. By 2024, this figure improved to 83%, but still fell short of the 95% target. Only 111 out of 147 infants were tested within six weeks, equating to 76% (70).

A 2016 qualitative study in Greater Accra, Ghana, interviewed 20 healthcare workers and 30 HIV-positive mothers. Findings showed that only 30% of HIV-exposed infants completed their first PCR test (71). A 2014–2016 cross-sectional study in Ruvuma Islands found that no HIV-exposed infants completed all EID tests: 39.5% received the 6-week PCR, 6.1% the second test, and 81.0% had rapid HIV testing (72). A study in Iringa District, Tanzania, from May to June 2023, involved 309 mothers with HIV-exposed infants. It found that 78.3% of infants received their first DNA PCR test within 6 weeks and 86.1% within 8 weeks, indicating a moderately high uptake of early infant diagnosis (73).

A July 2021 study in Ngombe, Tanzania, found 85% of HIV-exposed infants received 4–6 week PCR testing, higher than in many sub-Saharan countries, though gaps remain (66). A facility-based cross-sectional study was conducted in the Iringa region of Tanzania from December 2015 to February 2016. The study found that only 34.6% of infants received HIV PCR testing at 6–8 weeks after cessation of breastfeeding (74). A facility-based cross-sectional study in Dodoma, Tanzania (May–July 2023) enrolled 147 caregivers of HIV-exposed infants. Uptake of early infant HIV diagnosis was assessed, with 111 infants (76%) receiving HIV PCR testing at 6–8 weeks (65).

A cohort study in Khayelitsha (2014–2018) enrolled HIV-positive mothers and their newborns, testing infants at birth with PCR and following them up to 18 months. Uptake of the 6/10-week PCR was 77% under routine care, rising to 82% with tracing (75). A systematic review and meta-analysis conducted in Ethiopia in 2021, which included 19 studies from six regions, revealed that following the implementation of the Option B+ regimen, only about 64.8% approximately two-thirds of HIV-exposed infants were tested by 6 weeks of age (20). A cross-sectional study in Mekele, Ethiopia, reviewed 558 HIV-exposed infants; 62% received their first PCR test on time at 6–8 weeks (76).

Additionally, a comparative cross-sectional study conducted from January to December 2020 among 275 HIV-exposed infants in Dire Dawa and Harar showed that the uptake of early infant diagnosis was 64.2% in Dire Dawa and 52.7% in Harar city, respectively (77). A 2018 mixed-method study in West Shoa Zone, Ethiopia, analyzed 342 mother-infant pairs and found HIV PCR testing uptake at 4–6 weeks was 58.5%, far below the 95% national target (78). A retrospective study at Jimma University Specialized Hospital reviewed 225 HIV-exposed infants; 5.3% were HIV-positive, but only 6.7% tested at 6 weeks, highlighting delays and the need to strengthen PMTCT strategies (79).

2.6. Factors associated with the timeliness of early infant HIV diagnosis among HIV exposed infants

2.6.1 . Maternal sociodemographic factors

This cross-sectional study at Kisenyi Health Centre IV in Kampala, Uganda, from December 2016 to April 2017 involved 246 HIV-positive mother-baby pairs. A key factor associated with testing per Early Infant Diagnosis (EID) guidelines was maternal age over 30 years (69). A cross-sectional study in Iringa District, Tanzania (May–June 2023) enrolled 309 mothers with HIV-exposed infants. It found that 78.3% received their first DNA PCR test within 6 weeks and 86.1% within 8 weeks. Rural residence significantly reduced timely testing, exposing service access gaps outside urban settings (73). In July 2023, an analytical cross-sectional study conducted in Tanzania found that married caregivers were more likely to seek and utilize early diagnostic services for HIV in their infants. This indicates that marital status may significantly influence health-seeking behavior and access to essential health services for children at risk of HIV infection (70). Research conducted in Nigeria indicates that the factors associated with low uptake of early infant diagnosis (EID) services include young maternal age (64).

A 2022 study conducted in Mozambique demonstrated that lower maternal education significantly hinders early infant HIV testing uptake; specifically, mothers without formal education exhibited a 30–40% reduced likelihood of having their infants tested within the critical first two months compared to those with secondary education or higher (80). A prospective cohort study conducted in 2022 in Nairobi, Kenya, among 166 mother–infant pairs found that maternal age between 18 and 24 years was significantly associated with an early infant death due to HIV infection (81).In Tigray in 2019, a cross-sectional study was conducted on 350 exposed infants born to HIV seropositive mothers, showing that Higher proportions of HIV positive infants were from non-married mothers 46.1%), and not being married was a risk factor deemed to be statistically significant for infants contracting HIV (82). According to

research on Lost to Follow-up and Predictors Among HIV-Exposed Infants in Northwest Ethiopia, Infants whose mothers live in rural areas were at an AOR 3 times higher risk of lost to follow-up compared with infants whose mothers live in urban areas (83).

This cross-sectional study, conducted in Kericho County, Kenya, assessed early infant diagnosis (EID) service use among HIV-exposed infants. A total sample of 254 mother–infant pairs with Overall, only 29.5% of children utilized EID services, showing a low level of uptake in the county. Maternal education emerged as one of the strongest determinants of EID utilization (84). A cross-sectional study conducted in 2023 in selected healthcare facilities of Ruvuma Islands, Uganda, analyses secondary data from mother–infant pair records collected between January 2014 and December 2016 (72). The study found that being under the care of a single mother significantly increased the likelihood of not receiving the first DNA PCR test within six weeks (72). A retrospective cohort study conducted at the University of Gondar Comprehensive Specialized Hospital identified rural maternal residence as a significant predictor of loss to follow-up among HIV-exposed infants (85). Limited healthcare access and poor transportation in rural areas delay follow-up visits and infant HIV testing, hindering timely diagnosis and care. Infants of mothers with three or more children face higher loss to follow-up, as caregiving demands and resource constraints lead to missed appointments. Low birth weight also increases risk of delayed EID, since associated health complications often disrupt clinic attendance and timely initiation of prophylaxis or treatment (86).

A mixed-methods study in West Shoa Zone, Oromia Region, Ethiopia, reviewed 342 mother–infant pairs from 2014–2018 and conducted qualitative interviews with mothers and service providers. The study focused on HIV-exposed infants enrolled in PMTCT services and found that maternal education was a key determinant of Early Infant Diagnosis (EID) uptake. Mothers with secondary education or higher were more than twice as likely to have their infants tested early, underscoring education as a critical factor in improving EID service utilization (78).

2.6.2. Maternal Clinical Condition factors

In a retrospective cohort study conducted in Kembata-Tembaro Zone, Southern Ethiopia, several clinical and nutritional factors were identified as significant predictors of survival among HIV-infected children on ART, indirectly reflecting the timing of early infant diagnosis (EID). Children presenting with advanced WHO clinical stage III or IV had a markedly higher hazard of mortality, which is indicative of delayed diagnosis and late treatment initiation (87).HIV/TB co-infection was associated with significantly delayed ART initiation, reflecting the clinical complexities that postpone treatment. This delay potentially extends the window before infants receive timely HIV testing and care, increasing the risk of disease progression and transmission(49). Research conducted in a Myanmar retrospective cohort study involving record review of 1349 exposed infants shows that mothers not on ART before pregnancy were the risk factors for delayed or no sample collection (88).A mixed-method study was conducted in 2018 in West Shoa Zone, Oromia Region, Ethiopia, to assess early infant diagnosis of HIV. The study employed a retrospective review of four years of facility records supplemented by qualitative interviews, with a total sample of 342 mother–infant pairs as the target population. The overall uptake of HIV PCR testing at 6–8 weeks of age was 58.5% (78).

2.6.3 Infant factors

Research conducted in Nigeria indicates that the factors associated with low uptake of EID services include infant influences, such as the infant not receiving prophylaxis (64). A cross-sectional study design conducted among 558 HIV exposed infants in public hospitals of Mekelle City, North Ethiopia, in 2020, shows that being low birth weight and not receiving ARV prophylaxis were delayed infant diagnosis, as well as the overall HIV positivity (89) .A retrospective cohort study in the Oromia Liyu Zone, Amhara Region, Ethiopia, involving 376 HIV-infected children under five on antiretroviral therapy (2014–2019) found that the absence of isoniazid prophylaxis was significantly associated with higher mortality. Low birth weight was also independently associated with loss to follow-up among HIV-exposed infants. Low birth weight infants often have increased health complications that may affect the timing and frequency of clinic visits, resulting in delays in early HIV diagnosis and initiation of prophylaxis or treatment (86). An institution-based retrospective cohort study conducted at the University of Gondar Comprehensive Specialized Hospital from August 2013 to June 2018, involving 423 randomly selected mother–child pairs, identified underweight status as a

significant predictor of delayed infant diagnosis (86). In a retrospective cohort study of 399 newly diagnosed HIV-positive individuals in Adama Town, Ethiopia (September 2017–August 2021), being female was significantly associated with earlier ART initiation. This finding implies that prompt ART initiation in women of reproductive age can enhance prevention of mother-to-child transmission by improving maternal viral suppression, which is critical for reducing delays in early infant diagnosis (EID) of HIV infection and ensuring timely initiation of care for HIV-exposed infants (90). Research conducted on factors associated with the timely uptake of initial HIV virologic test among HIV-exposed infants attending clinics within a faith-based HIV program in Kenya; a cross-sectional study declared that infants who had not received prophylaxis to prevent vertical HIV transmission had significantly increased odds of a delayed initial test (91). A review of 558 HIV-exposed infants in Mekelle (2014–2017) found that 62% received timely HIV testing. Maternal antenatal care attendance and infant-feeding counselling were strongly associated with timely testing, while low birth weight and lack of ARV prophylaxis reduced the likelihood of prompt testing (76).

2.6.4. OBSTETRIC FACTORS

A study was conducted at King Edward VIII Hospital in South Africa from December 2018 to October 2019. It found that 73.3% of infants received HIV PCR testing at 10 weeks, largely due to an mHealth intervention, which posed barriers to follow-up testing (9).In South Gondar (2019–2021), the absence of PMTCT increased the hazard of infant HIV positivity over fivefold, strongly indicating a higher risk of delayed EID. Lack of PMTCT engagement severely disrupts the EID cascade, as mothers miss counselling, infant prophylaxis, and service linkage (92). Infants born to mothers with three or more children were more likely to be lost to follow-up (The increased caregiving demands and resource constraints in larger families can lead to missed appointments and delayed EID, as mothers may priorities other responsibilities over timely infant HIV testing and care (86). Home delivery significantly increases the risk of HIV positivity among exposed infants, with a sub-distribution hazard ratio of 4.1 times in a South Gondar retrospective study (2019–2021). This likely results from missed postnatal interventions and delayed linkage to early infant diagnosis services, highlighting the importance of facility-based births to improve timely HIV testing (93). An institution-based retrospective cohort study conducted at the University of Gondar Comprehensive Specialized Hospital from August 2013 to June 2018 assessed predictors of time to early infant diagnosis (EID) among 423 randomly selected mother–infant pairs. The study revealed that infants born

to mothers with three or more children had a significantly higher hazard of delayed EID compared to those born to mothers with fewer children (86). This finding suggests that higher parity may be associated with competing childcare demands. Such delays are critical in the context of HIV prevention and treatment, as timely diagnosis is essential for early initiation of antiretroviral therapy and improved survival outcomes (86). A cross-sectional study at Kericho County Referral Hospital found that only 29.5% of children utilized EID services, with place of delivery identified as a significant determinant of uptake. The study concludes that EID utilization remains low in Kericho County and highlights the importance of promoting institutional delivery to improve service uptake (84). A study conducted in Kenya reveals that the non-attendance of maternal antenatal care, a recent diagnosis of maternal HIV, and the absence of maternal antiretroviral therapy are significant factors leading to the delayed diagnosis of infants exposed to HIV (94). A systematic review and meta-analysis of 6,924 HIV-positive mothers and their exposed infants across 19 studies in six regions of Ethiopia found that the absence of antenatal care follow-up and home deliveries were associated with delays of 4.46 and 6.83 months, respectively, in obtaining early infant diagnosis (95). A cross-sectional review of 558 HIV-exposed infants in Mekelle public hospitals (2014–2017) found that 62% received timely HIV testing; infants of mothers who attended antenatal care, had low birth weight, or lacked ARV prophylaxis were less likely to be tested on time (76). An institution-based cross-sectional study conducted in 2018 across three public hospitals in Gambella, Ethiopia, involving 250 HIV-exposed children and their mothers, found that syphilis co-infection was a major risk factor, contributing to HIV infection and delaying the uptake of early infant diagnosis (96). According to research conducted at the Jimma facility, a cross-sectional study among infants in 2018 within the infant diagnosis service revealed that having fewer than four children significantly affected early HIV diagnosis in HIV-exposed infants (79). At Bishoftu Hospital (2006–2014), 66.7% of 936 HIV-exposed infants were tested at 6–8 weeks; 4.3% were HIV-positive. Home births, low maternal CD4, lack of PMTCT, and absence of antenatal/HIV care predicted delayed testing (97).

2.6.5. HEALTH SYSTEM-RELATED FACTORS

Between May 2013 and August 2016, 30 healthcare facilities in rural Malawi participated in a quantitative cluster randomized trial that enrolled pregnant women with HIV and infants exposed to the virus. The study found that 310 infants (29.0% of the total cohort of infants exposed to HIV) were unable to receive dried blood spots (98)In a 2021 study conducted in the Shewa Zone of central Ethiopia, researchers found that facilities providing mother-to-mother support programs enabled early infant diagnosis within six weeks of life among 96 participants (99). A study conducted in Western Oromia from March to April 2014 assessed early infant diagnosis (EID) among 349 HIV-exposed infants. It found that having a mother support group at the facility significantly improved early diagnosis. Mothers in these groups received valuable guidance and support, which helped ensure timely enrollment and testing for their infants (66).

Conceptual Framework with the timeliness of early infant HIV diagnosis among HIV exposed infants (HEI)

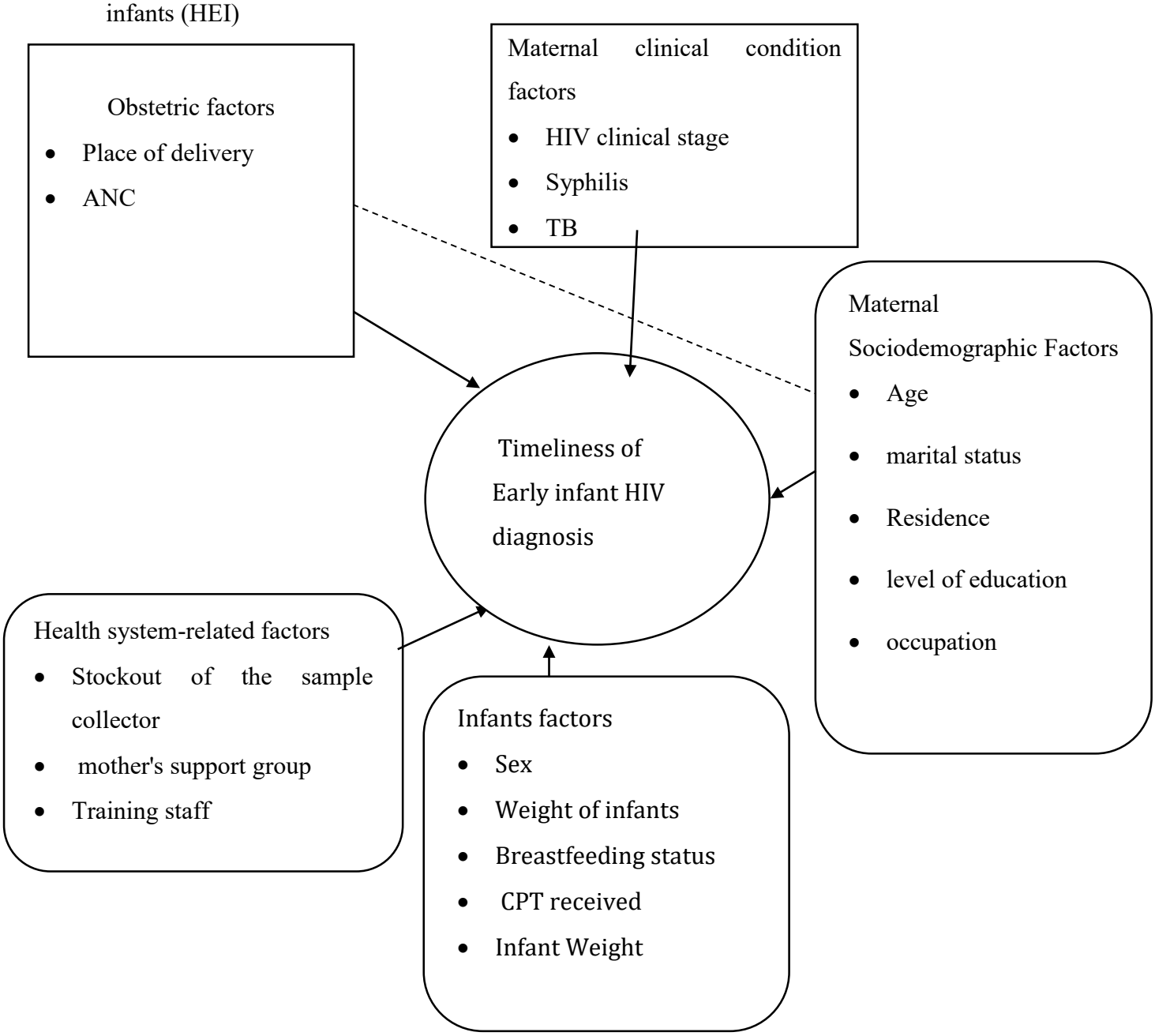

Figure 1: conceptual framework of timeliness of early infant HIV diagnosis among HIV exposed infants (HEI) in the public hospitals, Arsi Zone, in 2025

Source: This conceptual framework has been adapted from various literature sources based on research objectives (77, 100, 101).

3. OBJECTIVES

3.1 General Objective:

To assess the timeliness of early infant HIV diagnosis and identify associated factors among HIV-exposed infants in public hospitals of Arsi Zone, Southeast Ethiopia, 2025.

3.2 Specific objective

1. To determine the proportion of HIV-exposed infants with the first PCR sample collected within 6weeks of birth at selected public hospitals in Arsi Zone.

2. To identify factors associated with timely early infant HIV diagnosis (sample collection within 6 weeks) among HIV-exposed infants in Arsi Zone public hospitals.

4. METHODS

4.1 STUDY AREA

The study was conducted in three public hospitals in Arsi Zone, Southeast Ethiopia. Arsi Zone covers approximately 19,825 km² and has an estimated population of 3.9 million. About 11–13% of these people live in cities, while most live in rural areas. It is part of Oromia Regional State and is bordered by the Bale Zone to the south, the West Arsi Zone to the southwest, the East Shewa Zone to the northwest, the Afar Region to the north, and the West Hararghe Zone to the east (102). According to the official estimated population in mid-2022 reported to be 3,894,248. Assela is the capital of the Arsi Zone, which is located 159 km away from the capital city of Ethiopia (103). The zone has ten public hospitals; for this study, three were selected: Robe Didea General Hospital, Abomsa General Hospital, and Asella Referral and Teaching Hospital. From June 1, 2020, to June 1, 2025, a total of 478 HIV-exposed infants (HEIs) were enrolled across these facilities: 340 at Asella, 74 at Robe Didea, and 62 at Abomsa.

4.2 STUDY DESIGN AND PERIOD

A facility-based cross-sectional study was conducted using a retrospective chart review of HIV-exposed mother–infant pair logbooks from June 1, 2020, to June 1, 2025. Data collection took place from August 30 to September 30, 2025.

4.3. POPULATION

4.3.1 SOURCE POPULATION

All HIV-exposed infants and their corresponding mother–infant pairs who were tested for HIV and enrolled in PMTCT follow-up programs at public hospitals in Arsi Zone from June 1, 2020, to June 1, 2025.

4.3.2 STUDY POPULATION:

HIV-exposed tested infants and mother–infant pairs from the source population who met the eligibility criteria.

Inclusion Criteria:

HIV-exposed infants who received their first HIV DNA-PCR test and were recorded in the facility logbook.

Mother–infant pairs with complete documentation in the PMTCT cohort register, HIV DNA-PCR test logbook, DSD model of HIV care classification tool, and viral load registration logbook.

Exclusion Criteria:

Records are missing the infant's date of birth.

Records are missing the date of the first PCR sample collection.

Records are missing the maternal ART card number

4.4.1 Sample Size Determination

For the first objective (proportion):

The sample size was calculated using a single population proportion formula:

$$n = \frac{Z^2 .p.(1-p)}{d^2}$$

Where: n = the minimum required sample size

Z = the cut-off value of the normal distribution at 95% confidence level (CL)

α = the significance level = 0.05

P = the estimated proportion of early infant diagnosis in South Ethiopia, west shoe conducted in 2020, p-value =58.5% (78).

d = precision required on either side of the proportion (5%) = 0.05

By inserting into the formula

$$\frac{1.96^2 \cdot 0.585 \cdot (1 - 0.585)}{0.05^2}$$

$$n \approx \frac{3.8416 \cdot 0.585 \cdot 0.415}{0.0025}$$

$$n \approx \frac{0.933}{0.0025} \approx 373$$

373 by adding 11% for incomplete data and lost cards, the sample size was 414

For the second objective (factors):

Sample size was calculated using Epi Info™ StatCalc with parameters: 95% CI, 80% power. The largest sample size from key variables was 116 (Table 1).

Table 1: The sample size for the second objective on the timeliness of early infant HIV diagnosis among HIV-exposed infants in public hospitals of Arsi Zone, 2025

Variables	P1 =% Outcome among exposed	P2=% Outcome among unexposed	OR	Sample size with a 10% non-response rate	Reference
Breastfeeding status	52.5%	30%	0.42	116	(20)
Absence of PMTCT service	32.3%	10%	4.6	116	(104)
Place of delivery	34.8%	10%	4.8	89	(105)

A final sample size of 414 mother-infant pairs, determined from the calculations for the study's first primary objective, was employed for this research, thereby ensuring robust and statistically powered findings across all subsequent analyses.

4.4.2 . SAMPLING PROCEDURE

Out of the ten public hospitals in Arsi Zone, three facilities, Asella Referral and Teaching Hospital, Robe Didea General Hospital, and Abomsa General Hospital, were selected using simple random sampling (lottery method) to ensure geographic and service-level representativeness. Within each hospital, all HIV-exposed infant records registered between June 1, 2020, and June 1, 2025, were screened for completeness, with exclusions applied to records missing essential variables (infant's date of birth, date of first PCR sample collection, or maternal ART card number) or lost during follow-up. Proportional quotas were then allocated based on caseload, yielding final samples of 300 records at Asella Referral and Teaching Hospital after 30 logbooks were excluded, 62 records at Robe Didea General Hospital after 9 logbooks were excluded, and 52 records at Abomsa General Hospital after 10 logbooks were excluded from the initial 62. To achieve these quotas, consecutive non-probability sampling of sequentially registered complete records was employed until the required numbers were fulfilled. With sampling fractions of 88.2% at Asella Referral and Teaching Hospital, 83.8% at Robe Didea General Hospital, and 83.9% at Abomsa General Hospital, the study effectively approached a near-census of eligible cases, thereby minimizing selection bias, maximizing data utilization, and preserving chronological representativeness for robust assessment of early infant HIV diagnosis timeliness.

4.4.3 . SAMPLING PROCEDURE

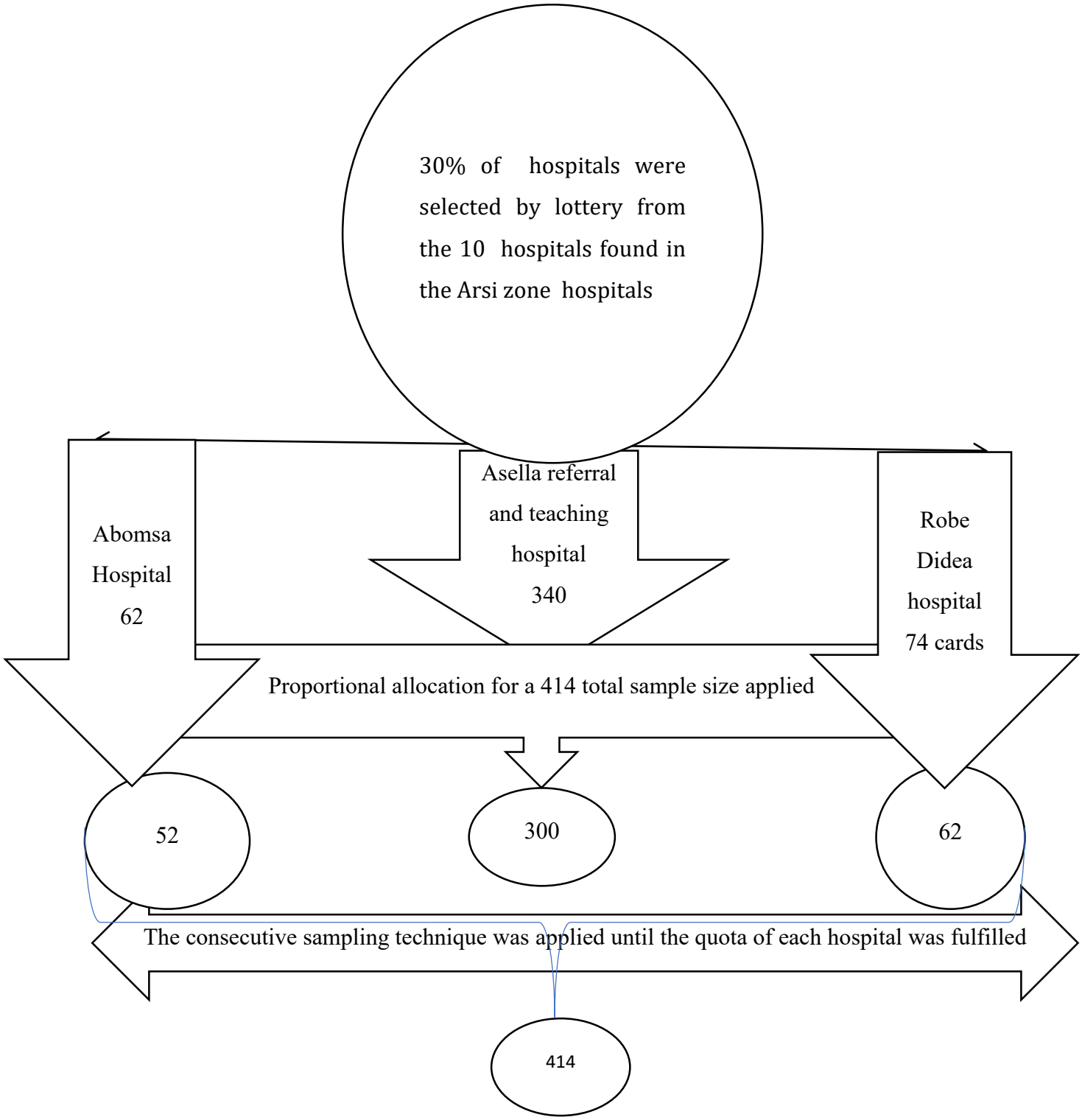

Figure 2: final sample size diagram on timeliness of early infant HIV diagnosis among HIV-exposed infants (HEIs) at a public hospital in the Arsi zone, southeast Ethiopia, 2025

4.5 STUDY VARIABLE

4.5.1 DEPENDENT VARIABLE

Timeliness of Early infant HIV diagnosis

4.5.2 INDEPENDENT VARIABLES

- Maternal sociodemographic factors
  - Age
  - marital status
  - Residence
  - level of education
  - occupation
- Infant factors
  - Sex
  - Weight of the infant
  - Breastfeeding status
  - The infant received CPT
- Maternal clinical condition factors
  - HIV stage
  - Syphilis
  - TB
- Obstetric factors
  - Place of delivery
  - ANC

- Healthcare-related system factors:
- stock out of sample collectors
- Training staff with EID sample collection skills
- a mother support group at the facility.

4.6. OPERATIONAL DEFINITIONS

Early Infant HIV Diagnosis refers to the early detection of HIV infection in infants born to HIV-positive mothers (26, 27, 106).

Timeliness of Early infant HIV diagnosis is defined as sample collection for the first PCR test for virological testing using PCR HIV DNA within 6 weeks of birth (107).

First PCR tested = sample collected for first PCR test (78).

Outcome variable - Timeliness of Early infant HIV diagnosis

Timeliness ~measured in weeks

Dichotomised into
Early diagnosis - HIV exposed Infant for whom a whole blood (DBS sample) was collected for a test before or at 6 weeks of age to confirm HIV status by DNA-PCR (78).

Late diagnosis -For an HIV-exposed infant for whom whole blood (DBS sample) was collected for a test after 6 weeks of age to confirm HIV status by DNA-PCR, and after 6 weeks of breastfeeding cessation, using an antibody test or by DNA-PCR test (78).

4.7 DATA COLLECTION TOOL AND PROCEDURES

Data collection was conducted over one month by seven trained midwives using the Kobo Toolbox platform. Before abstraction, the midwives participated in a structured training session that covered the objectives of the study, standard procedures for chart review, orientation on the structured checklist, and ethical principles in handling clients' records. The training emphasised consistency in variable coding, accurate transcription of dates, and minimisation of subjective interpretation. The structured checklist, prepared in English, was developed based on national and international HIV-exposed infant diagnosis and treatment guidelines and was organised into different domains.

The infant domain included variables such as date of birth of exposed infant and date of first PCR sample collected from exposed infants, while the maternal domain captured socio-demographic and clinical information abstracted from the Differentiated Service Delivery (DSD) model of HIV care, including maternal age, marital status, residence, education, occupation, HIV stage, and co-morbidities such as syphilis and tuberculosis. Obstetric and antenatal care data, including ANC attendance and place of delivery, were reviewed from cohort registration records.

While the health-related system domain (such as stock-outs, sample collectors and staff training on EID) was obtained through direct observation of facility records, stock ledgers and via a brief interview with the PMTCT focal person, supervision was provided by the principal investigator and hospital HIV program coordinators, who conducted daily spot-checks of completed checklists, verified random entries against source documents, and provided immediate feedback to data collectors. These measures ensured adherence to standardised procedures and minimised variability across hospitals.

4.8 DATA QUALITY MANAGEMENT

To ensure data quality, a one-day orientation was provided to all data collectors, focusing on uniform record review techniques, checklist completion, and error prevention. The checklist was pre-tested among 5% (21 records) of the total sample size at Bokoji hospitals to assess the clarity, consistency, and feasibility of data collection. The pretest confirmed that the tool was appropriate for capturing the required information, and minor modifications were made to improve wording and alignment with study objectives, such as the chronological order of the

checklist. Reliability was assessed by comparing duplicate abstractions of the same records by two midwives, with discrepancies discussed and resolved to achieve consensus.

Continuous supervision and daily monitoring were conducted throughout data collection. Supervisors reviewed completed checklists each evening, flagged inconsistencies, and provided corrective guidance. To control bias, selection bias was minimised through proportional allocation across hospitals, information bias was reduced by relying on standardised hospital registers, and recall bias was avoided since the study exclusively used secondary data sources

4.9 DATA ANALYSIS

Data from 414 records were entered into KoboToolbox, checked for inconsistencies, duplicates and outliers, then exported to IBM SPSS version 27 for final cleaning, yielding a complete-case dataset. The primary outcome, timeliness of Early Infant HIV Diagnosis, was defined as a dichotomous variable per the Ethiopia National HIV Guideline 2023: early diagnosis = Yes (sample collected for first virological PCR test ≤45 days from date of birth, coded as 1); delayed diagnosis = No (sample collected for first virological PCR test at >45 days, coded as 0). All factors (socio-demographic, maternal clinical/obstetric, infant and health service factors) were categorised or dichotomised as appropriate. Descriptive statistics were computed first (frequencies/percentages for categorical variables; means ± SD for continuous variables), with results presented in tables/figures and cross-tabulations by EID timeliness. Bivariate screening logistic regression to estimate crude odds ratios (COR) with 95% CIs; variables with $p < 0.25$ and those without multicollinearity were retained for multivariable modelling. Forward stepwise binary logistic regression was then applied to identify independent predictors, with clinically important variables (e.g., maternal age, residence) forced into the final model if excluded by the stepwise procedure; adjusted odds ratios (aOR), 95% CIs and p-values were reported. Model assumptions were verified: missing data handled by complete-case analysis, multicollinearity absent (all VIF < 1.99), linearity in the logit confirmed for continuous variables, and the Hosmer-Lemeshow goodness-of-fit test indicated excellent fit ($p = 0.343$). All analyses were two-sided with statistical significance set at $p < 0.05$ and a 95% confidence level.

4.10 ETHICAL CONSIDERATION

Ethical approval for this study was obtained from the Arsi University, College of Health Sciences Institutional Ethics Research Committee (ERC) under protocol number AU/CoHS/R/204/2025, date September 02, 2025. Following approval, the College of Health Sciences issued a formal letter of cooperation to the relevant authorities, outlining the objectives of the study and requesting their support and commitment. Permission to review patient charts was granted by the medical directors of the respective hospitals. As the study was conducted in the form of a retrospective chart review, no individual patients were subjected to any direct risk. To ensure confidentiality, personal identifiers were not recorded, and data collectors were explicitly instructed not to include participants' names on the checklist. All procedures adhered to institutional ethical standards and respected the principles of privacy and confidentiality.

4.11 Dissemination plan

Study findings will be disseminated to key stakeholders, including the Arsi Zone Health Department, the Oromia Regional Health Bureau, the Ethiopian Ministry of Health (HIV/AIDS Prevention and Control Office), and Arsi University College of Health Sciences.

- An executive summary and full report will be submitted officially within one month of the thesis defence.
- Oral and poster presentations will be delivered at regional/national HIV review meetings, Ethiopian Public Health Association annual conferences, and Arsi University research symposia.
- The thesis will be uploaded to Arsi University's institutional repository for open access.
- A manuscript reporting the main results will be prepared and submitted to a reputable peer-reviewed journal (targeting BMC Infectious Diseases, PLoS ONE, or Ethiopian Journal of Health Sciences) within three months of defence.
- Policy briefs and infographics summarising key findings and recommendations will be shared with implementing partners (e.g., ICAP-Ethiopia, USAID) to inform PMTCT/EID program improvements in the zone.

5. RESULTS

5.1. SOCIO-DEMOGRAPHIC CHARACTERISTICS AMONG HIV-EXPOSED INFANTS

The study analysed 414 maternal-infant pairs, with most mothers aged 35 years or older (39.4%0). The majority resided in rural areas (53.1%). By marital status, 68.6% were married, and housewives accounted for 36.2% of participants. The largest educational group was elementary level (52.9%) (Table 2).

Table 2: Socio-demographic characteristics among HIV-exposed infants (HEIs) in public hospitals, Arsi Zone, 2025 (n = 414)

characteristics	Categories	Frequence(N)	Percentage (%)
Maternal age	< 25 years	124	30.0
	≥ 35 years	163	39.4
	25–34 years	127	30.7
Residence	Urban	194	46.9
	Rural	220	53.1
Marital status	Single	28	6.8
	Widowed	62	15.0
	Divorced	40	9.7
	Married	284	68.6
Maternal occupation	Private	67	16.2
	Daily Workers	73	17.6
	Housewife	150	36.2
	Government Employees	124	30.0
Maternal education	No formal education	29	7.0
	Elementary	219	52.9
	Secondary	45	10.9
	Higher	121	29.2

5.2. Maternal Clinical Condition and Obstetric Characteristics

Nearly half of the mothers (49.5%) delivered at the same health facility where they received care, though home delivery remained common (37.2%). Most mothers (51.7%) had WHO HIV clinical stage III. Testing for TB and syphilis was incomplete, with only 46.9% and 47.1% tested, respectively (Table 3).

Table 3: Maternal clinical condition and obstetric characteristics among HIV-exposed infants (HEIs) in public hospitals, Arsi Zone, 2025 (n = 414)

characteristics	Categories	Frequence(N)	Percentage (%)
Place of delivery	At Home	154	37.2
	At Other Health Facility	55	13.3
	At the Same Health Facility	205	49.5
ANC follow-up	Yes	258	62.3
	No	156	37.7
HIV clinical stage	Stage Four	28	6.8
	Stage Three	214	51.7
	Stage Two	44	10.6
	Stage One	128	30.9
Tested for TB	Yes	194	46.9
	No	220	53.1
Tested for syphilis	Yes	195	47.1
	No	219	52.9

5.3. INFANTS' RELATED AND HEALTH-RELATED SYSTEM CHARACTERISTICS AMONG HIV-EXPOSED INFANTS (HEI)

The infant population comprised mostly females (70.3%), with nearly half exclusively breastfed (48.6%) and almost all (94.2%) having normal birth weight. While trained staff availability was excellent (97.6%) and supply stock-outs were rare (1.9%), support group participation was low (30.9%), and cotrimoxazole (CPT) coverage was nearly balanced (48.8% vs. 51.2%) (Table 4).

Table 4: Infant-related and health system characteristics among HIV-exposed infants (HEIs) in public hospitals, Arsi Zone, 2025 (n = 414)

characteristics	Categories	Frequence(N)	Percentage (%)
Sex	Male	123	29.7
	Female	291	70.3
Breastfeeding status	MBF	140	33.8
	ERF	73	17.6
	EBF	201	48.6
Infants weight	≥ 2.5 kg	390	94.2
	< 2.5 kg	24	5.8
CPT	Yes	202	48.8
	No	212	51.2
Support group present	Yes	128	30.9
	No	286	69.1
Availability of trained staff at	Yes	404	97.6
enrollment	No	10	2.4
Stock-out of the sample collector	Yes	8	1.9
	No	406	98.1

5.4. Proportion of Timeliness of Early Infant HIV Diagnosis

Among the 414 HIV-exposed infants enrolled (300 from Asella referral and Teaching Hospital, 62 from Robe Didea General Hospital, and 52 from Abomsa General Hospital), only 61.6% (95% CI: 56.8–66.2%) had their first PCR sample collected within the recommended 6 weeks from the date of birth. Timeliness differed significantly across facilities (p=0.002): Asella referral and Teaching Hospital recorded the highest rate at 68.3%, followed by Robe Didea General Hospital (51.6%) and Abomsa General Hospital (44.2%). Overall, GeneXpert was used for 69.9% of tests (predominantly at Asella referral and Teaching Hospital), yielding a median laboratory turnaround time of just 1 day, compared with 54 days for conventional dried blood spot PCR (p<0.001). Results for 73.0% of infants (302/414) were returned in hard-copy format. Fifteen infants (3.6%) were confirmed HIV-positive; only 2 (16.7%) were diagnosed within the early 6-week window. Among 15 HIV exposed infants, only 13 (86.7%) were linked to the ART clinic within 7 days, which means they received points of care(Figure 4).

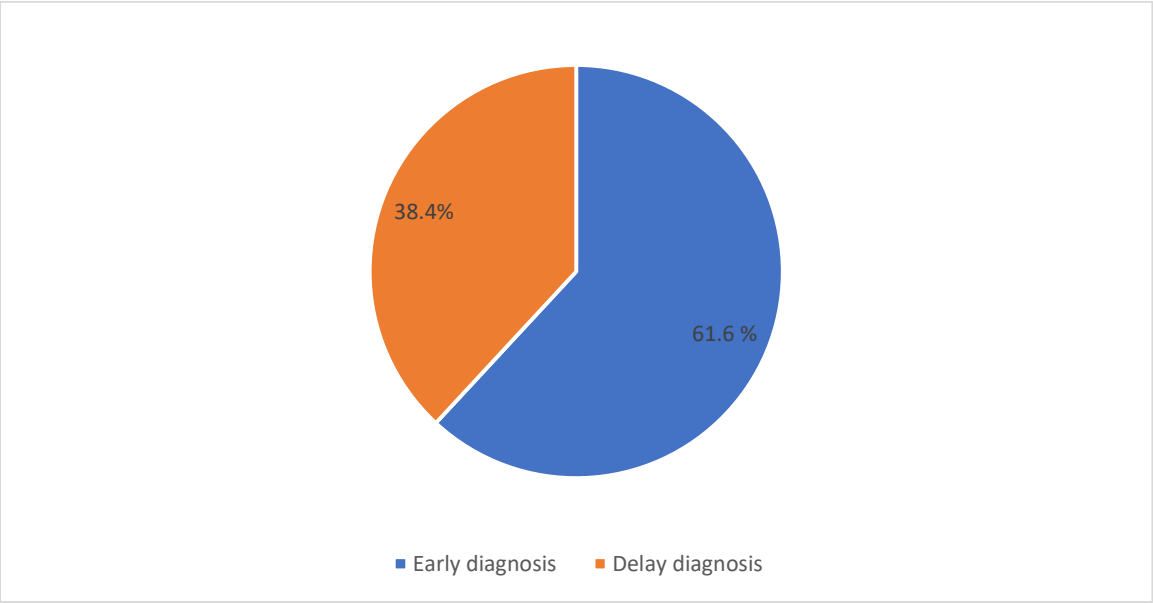

Figure 3: The proportion of timeliness among HIV-exposed infants (HEIs) in the public hospital, Arsi Zone,2025 (n = 414)

The proportion of timeliness of early infant HIV diagnosis related each hospital

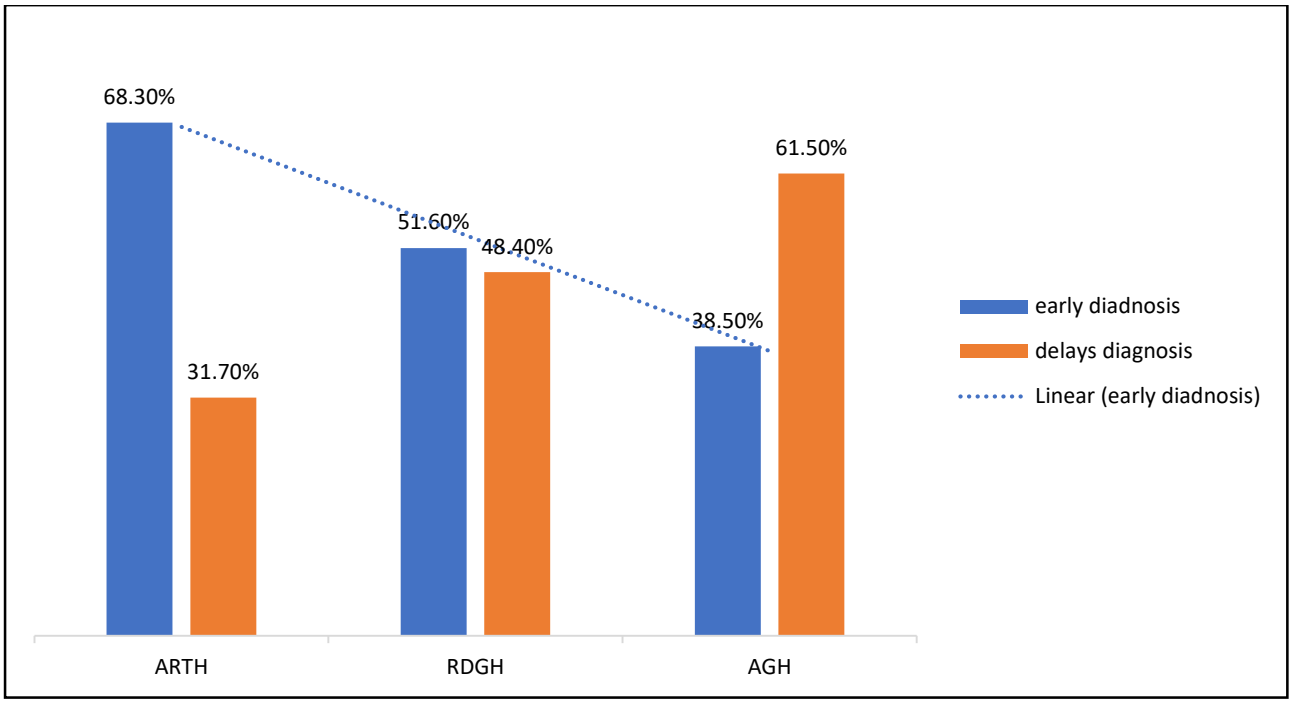

Figure 4 : Proportion of timeliness by hospital among HIV-exposed infants (HEIs) in public hospital, Arsi zone,2025 (n = 414)

(Asella Referral and Teaching Hospital (ARTH)

Robe Didea General Hospital (RDGH), and

Abomsa General Hospital (AGH)

5.5. Socio-demographic characteristics associated with the timeliness of early infant HIV"

Early infant diagnosis was most frequent among mothers aged 25–34 years (80.3%), urban residents (80.4%), married women (66.9%), daily workers (75.3%), and those without formal education (72.4%) (Table 5).

Table 5: Timely diagnosis by socio-demographic characteristics of HIV exposed infants (HEIs) in public hospital, Arsi Zone, 2025 (n = 414)

Characteristic		Diagnosed status	
	Categories	Early diagnosed n (%)	Delay diagnosed n (%)
Maternal age	<25 years	40 (32.3%)	84 (67.7%)
	25–34 years	102 (80.3%)	25 (19.7%)
	>35 years	113 (69.3%)	50 (30.7%)
Residence	Rural	99 (45.0%)	121 (55.0%)
	Urban	156 (80.4%)	38 (19.6%)
Marital status	Single	12 (42.9%)	16 (57.1%)
	Widowed	42 (67.7%)	20 (32.3%)
	Divorced	11 (27.5%)	29 (72.5%)
	Married	190 (66.9%)	94 (33.1%)
Occupation	Private	44 (65.7%)	23 (34.3%)
	Daily workers	55 (75.3%)	18 (24.7%)
	Housewife	78 (52.0%)	72 (48.0%)
	Government employees	78 (62.9%)	46 (37.1%)
Education	No formal education	21 (72.4%)	8 (27.6%)
	Elementary	134 (61.2%)	85 (38.8%)
	Secondary	23 (51.1%)	22 (48.9%)
	Higher	77 (63.6%)	44 (36.4%)

5. 6. MATERNAL CLINICAL AND OBSTETRIC CHARACTERISTICS RELATED TO TIMELY EID"

Early infant diagnosis was significantly more common among mothers who delivered at the same health facility (84.9%) and those who attended ANC follow-up (77.1%). HIV stage showed no major differences across categories, while TB and syphilis testing were modestly associated with higher rates of early diagnosis(Table 6).

Table 6: Proportion of maternal and obstetric characteristics among HIV-exposed infants (HEIs) in public hospital, Arsi Zone,2025 (n = 414)

characteristics	Category	Diagnosed status	
		Early diagnosed n (%)	Delay diagnosed n (%)
place of delivery	At Home	49 (31.8%)	105 (68.2%)
	At Other HF	32 (58.2%)	23 (41.8%)
	At the Same HF	174 (84.9%)	31 (15.1%)
ANC follow-up	No	56 (35.9%)	100 (64.1%)
	Yes	199 (77.1%)	59 (22.9%)
HIV clinical stage	Stage I	78 (60.9%)	50 (39.1%)
	Stage II	29 (65.9%)	15 (34.1%)
	Stage III	127 (59.3%)	87 (40.7%)
	Stage IV	17 (60.7%)	11 (39.3%)
Tested for TB	No	129 (58.6%)	91 (41.4%)
	Yes	126 (65.0%)	68 (35.1%)
Tested for syphilis	No	131 (59.8%)	88 (40.2%)
	Yes	124 (63.6%)	71 (36.4%)

5.7. Proportion of infant and health-system characteristics related to the timeliness of Early Infant Diagnosis (EID) among HIV-exposed infants

Among HIV-exposed infants, delayed diagnosis was more common in males, those with mixed breastfeeding, low birth weight, and absence of antenatal or support services(Table 7).

Table 7: proportion of infants and health-related system-related among HIV-exposed infants (HEIs) in public hospital, Arsi Zone,2025 (n = 414)

characteristics		Diagnosis status	
	Categories	Early diagnosed n(%)	Delay diagnosed n (%)
Sex	Male	55 (44.7%)	68 (55.3%)
	Female	200 (68.7%)	91 (31.3%)
Breastfeeding status	MBF	42 (30.0%)	98 (70.0%)
	ERF	53 (72.6%)	20 (27.4%)
	EBF	160 (79.6%)	41 (20.4%)
Infants weight	<2.5 kg	11 (45.8%)	13 (54.2%)
	≥2.5 kg	244 (62.6%)	146 (37.4%)
CPT	No	129 (60.8%)	83 (39.2%)
	Yes	126 (62.4%)	76 (37.6%)
Absent trained staff	Yes	83 (63.4%)	48 (36.6%)
	No	172 (60.8%)	111 (39.2%)
Stock-out of the sampler collector	Yes	7 (87.5%)	1 (12.5%)
	No	248 (61.1%)	158 (38.9%)
Support group absent	Yes	83 (63.4%)	48 (36.6%)
	No	172 (60.8%)	111 (39.2%)

5.8. Factors Associated with Timeliness of Early Infant HIV Diagnosis

Of 17 variables assessed, 12 entered the multivariate model ($p < 0.25$); 5 were excluded during adjustment, leaving 6 factors independently associated with lower odds of timely EID (PCR sample within six weeks) (Table 8).

Table 8. Bivariate and multivariable logistic regression analysis of factors associated with timely EID among HIV-exposed infants (HEIs) in Arsi Zone, 2025 (n = 414)

Characteristic		Diagnosed status	
	Categories	Bivariable COR (95% CI)	multivariate OR (95% CI)
Marital status	Single	0.371 (0.169 - 0.816)*	0.119 (0.028 - 0.502)**
	Widowed	1.039 (0.578 - 1.869)	1.027 (0.373 - 2.824)
	Divorced	0.188 (0.090 - 0.392)*	0.103 (0.025 - 0.427)**
	Married	1	1
Occupation	Private	1.128 (0.606 - 2.102)	1.745 (0.556 - 5.476)
	Daily	1.802 (0.945 - 3.435)	1.241 (0.421 - 3.658)
	Housewife	0.639 (0.393 - 1.038)	1.609 (0.723 - 3.582)
	Governments employes	1	1
Residence	Urban	1	1
	Rural	0.199 (0.128 - 0.310)*	0.124 (0.056 - 0.276)**
ANC follow-up	Yes	1	1
	No	0.166 (0.107 - 0.257)*	0.163 (0.076 - 0.349)**
Place of delivery	At Home	0.083 (0.050 - 0.139)*	0.083 (0.033 - 0.211)**
	At other HF	.248 (0.128 - 0.479)*	0.324 (0.094 - 1.114)
	At the same HF	1	1
Breastfeeding status	MBF	0.110 (0.067 - 0.181)*	0.126 (0.049 - 0.321)**
	ERP	0.679 (0.366 - 1.260)	1.560 (0.519 - 4.687)
	EBF	1	1

Sex of infant	Male	1	1
	female	0.368 (0.239 - 0.568)*	0.792 (0.287 - 2.184)
Weight of infants	> 2.5 kg	1	1
	< 2.5 kg	0.506 (0.221 - 1.160)	0.380 (0.084 - 1.732)
Maternal age	< 25 years	0.117 (0.066 - 0.208)*	0.043 (0.015 - 0.121)**
	> 35 years	0.554 (0.320 - 0.960)*	0.257 (0.103 - 0.641)**
	25 – 34 years	1	1
TB tested	Yes	1	1
	No	0.765 (0.513 - 1.140)	0.617 (0.235 - 1.617)
Lack of trained staff	yes	5.780 (0.725 - 46.068)	3.415 (0.245 - 47.674)
	No	1	1
Stock out happens	Yes	4.460 (0.544 - 36.592)	0.535 (0.047 - 6.085)
	No	1	1

CI = Confidence Interval; AOR = Adjusted Odds Ratio. * denotes bivariable significance ($p < 0.05$, CI excludes 1), ** denotes multivariable significance ($p < 0.05$). Outcome was early diagnosis (coded 1) vs. delayed (coded 0), with early diagnosis as reference. Model fit was acceptable (Hosmer–Lemeshow $p = 0.343$); no multicollinearity (VIF < 1.909, tolerance > .524). Of 17 variables, 12 entered the multivariable model ($p < 0.25$ in bivariable), including MBF (mixed breastfeeding), EBF (exclusive breastfeeding), ERF (exclusive replacement feeding), and HF (health facility).

6. Discussion

This facility-based retrospective study of 414 HIV-exposed infants in three public hospitals of Arsi Zone, Southeast Ethiopia, assessed the timeliness of early infant diagnosis (EID) via HIV DNA-PCR testing and found that only "61.6% (95% CI: 56.8–66.2%) of infants had their first sample collected for PCR DRN test within the recommended 6 weeks, substantially below the WHO/UNAIDS target of ≥95%. Timeliness varied by facility, with Asella referral and Teaching Hospital achieving 68.3%, Robe Didea General Hospital 51.6%, and Abomsa General Hospital 44.2%, likely reflecting differences in PMTCT infrastructure, urban accessibility, and rapid testing availability. The uptake of the GeneXpert platform was high at 69.9%, dramatically reducing median laboratory turnaround time to 1 day compared with 54 days for conventional dried blood spot (DBS) PCR, although results were predominantly returned in hard-copy format for 73% of infants, potentially delaying caregiver notification. Among the cohort, 15 infants (3.6%) were confirmed HIV-positive, but only 2 were diagnosed within the 6-week window, highlighting critical gaps in early detection. Of these 15 HIV-positive infants, around 13 (86.7%%) were linked to the ART clinic within 7 days, reflecting timely provision of point-of-care services. Multivariable analysis identified several independent factors of delayed first PCR sampling, including rural maternal residence, home delivery, lack of antenatal care (ANC), maternal age under 25 years and over 35 years, mixed breastfeeding practices, and non-married maternal status, both being single and divorced.

The study found that only 61.6% of HIV-exposed infants in Arsi Zone had their first HIV DNA-PCR sample collected within the recommended 6 weeks, leaving nearly four in ten infants delayed. This finding was moderately higher than reports from several sub-Saharan African settings, including peri-urban Khayelitsha, South Africa (75), Nigeria (40.9 %,2023) (64), in Kenya, 33.5% in 2021 (68), and again in Kenya, 42.4% in 2017 (108), and including some parts of Ethiopia, such as the West Shoa Zone (58.5% in 2020)(78), Harar City (52.7%) (77), Jimma 6.7% (79). This may reflect improved PMTCT implementation, wider applicability of GeneXpert availability within these five years in those hospitals, especially Assela referral and teaching Hospital, and enhanced maternal follow-up in the Arsi Zone.

However, the first HIV DNA-PCR sample collected for HIV exposed infants for EID for HIV infection remains substantially lower than in high-income countries such as the United Kingdom, 90% (109), Norway, 92% (57), and the Netherlands, 90%(53). The first HIV DNA-PCR sample collected for HIV exposed infants for EID for HIV infection in the Arsi Zone was substantially lower, reflecting differences in health system infrastructure, universal testing policies, and laboratory capacity, where strong health systems, universal early testing policies, and reliable laboratory networks ensure prompt diagnosis and linkage to care in high-income countries. Similarly, even in some African countries, including peri-urban Khayelitsha, South Africa, 76 % (75), Tanzania (76% (70), Malawi (71.6%) (110), (79.0%) Kenya (91), Ethiopia, the national average a 64.8% (20), and even within Ethiopia, higher proportions have been reported in Tigray (83.7%) (111), in Bishoftu hospital (66.7 %) (97). The lower uptake in the Arsi Zone may be attributed to health system constraints, staff shortages, geographic barriers, security problems in the zone and sociocultural factors such as stigma and limited maternal health literacy. Unlike Tigray, where NGO support and strong programmatic monitoring have enhanced service delivery, Arsi Zone's weaker infrastructure likely contributes to reduced EID performance.

Infants born to mothers aged <25 years were dramatically less likely to receive timely EID than those born to mothers aged 25–34 years (over 95% lower odds after adjustment). Mothers aged >35 years were also substantially less likely to achieve timely testing (approximately 74% lower odds). These results highlight that both very young and older mothers face heightened barriers (adolescent mothers likely due to stigma, limited autonomy, and poorer service access; older mothers possibly due to competing family responsibilities and lower perceived risk), emphasising the need for age-specific counselling, youth-friendly services, and targeted support for high-parity women. These findings align with studies which also report age-related disparities in first PCR test timeliness (64, 69, 81, 112, 113, 114) However, this finding that maternal age was linked to lower odds of timely EID contrasts with studies (25, 69). This difference likely reflects contextual realities in Arsi Zoneolder mothers here may face competing responsibilities and weaker follow-up, while younger mothers benefit from recent PMTCT counselling and stronger health system support.

Infants born to single or divorced mothers were substantially less likely to receive timely EID compared with those born to married mothers. After adjustment, single mothers had nearly 90% lower odds of timely testing, while divorced mothers had more than 95% lower odds. Widowed mothers showed no meaningful difference from married mothers. These findings indicate that lack of a current partner, whether due to never marrying or marital dissolution, poses a major barrier to early infant testing, likely through reduced social support, economic constraints, and greater stigma, underscoring the need for targeted outreach and peer support for unmarried HIV-positive mothers. These findings highlight the need for targeted counselling and psychosocial support for mothers without partners, consistent with evidence from East Africa (56, 115), Tanzania (70), Uganda (72) and Addis Ababa (116). However, this finding was contradicted by a study conducted(28, 76). This finding that residence was associated with lower odds of timely EID contradicts reports from other settings where urban residence facilitated early testing. This difference may reflect contextual realities in Arsi Zone: rural mothers often benefit from strong community health worker follow-up and closer ties to local facilities, while urban mothers may face overcrowded clinics, longer waiting times, or competing work responsibilities that delay infant visits. Variations in health system organization and definitions of "timely EID" across studies may also explain the discrepancy.

Infants born to mothers who had no ANC follow-up were far less likely to receive timely EID than those whose mothers attended ANC. After adjustment, lack of ANC attendance was associated with approximately 84% lower odds of testing within 6 weeks. This strong association underscores that ANC serves as the primary gateway for education, counselling, and linkage to EID services; mothers who miss ANC are effectively disconnected from the PMTCT cascade, highlighting the need for intensified community outreach and active tracing of pregnant women who do not attend clinics. This result is consistent with evidence from a study conducted in Kenya (68). In North Ethiopia, at the public hospitals of Mekelle City, (76) In Ethiopia, the national average, a systematic review (20).

Infants delivered at home were dramatically less likely to receive timely EID compared with those delivered at the study health facilities (nearly 92% lower odds after adjustment). Delivery at other health facilities also reduced the likelihood of timely testing, though to a lesser extent. These findings demonstrate that birth within the study facilities provides the strongest opportunity for immediate registration, counselling, and sample collection in the EID cascade. Home deliveries and transfers from other facilities represent critical missed opportunities,

reinforcing the urgent need to promote institutional delivery, establish maternity waiting homes, and implement active postnatal tracing for all home births. This finding is supported by a study conducted across low- and middle-income countries (LMICs)(117), in Ethiopia: the national average, a systematic review (17, 20, 91, 93, 118), in South Gondar public hospitals, Northwest Ethiopia (93).

Infants from rural areas were substantially less likely to receive timely early infant diagnosis compared with their urban counterparts. After adjustment, rural residence was associated with nearly 88% lower odds of first HIV DNA-PCR testing within 6 weeks. This stark disparity reflects the multifaceted barriers faced by rural mothers, including long travel distances to health facilities, high transportation and opportunity costs, poor road infrastructure, and limited access to functional health posts. In addition, rural mothers often have reduced exposure to health education and ANC counselling, further delaying timely presentation for EID. These findings are consistent with prior studies in the Arsi Zone and other low-resource settings, which demonstrate that geographic isolation and structural health system limitations are major determinants of delayed HIV testing in infants. The evidence strongly supports the urgent need for decentralised testing, community-based sample collection, and transport support to improve timely first PCR sample collection and strengthen the EID cascade in rural populations. This was supported by the study (73, 86) . However, this was not supported by a study conducted (49, 52, 73, 76, 119)

Infants on mixed breastfeeding were far less likely to receive timely EID compared with those exclusively breastfed (approximately 87% lower odds after adjustment). Replacement feeding showed no significant difference from exclusive breastfeeding. This finding strongly suggests that mothers practising mixed feeding may face greater stigma, reduced counselling adherence, or lower engagement with health services, while those committed to exclusive breastfeeding are more likely to remain connected to PMTCT programs. It reinforces the importance of intensive, non-judgmental counselling to support exclusive breastfeeding and maintain mother–infant pairs within the care cascade. This finding is consistent with evidence from prior studies (76).

Limitations and Strengths of the Study

Limitations

This study faced several context-specific limitations. First, reliance on retrospective medical records introduced the possibility of incomplete or missing data, particularly regarding infant testing dates, maternal ART initiation, and counselling documentation. Such gaps may have led to information bias and reduced the precision of associations. Second, inconsistencies in documentation, such as infant age at testing or maternal service uptake, could have resulted in inadvertent misclassification of "timely" versus "delayed" early infant diagnosis (EID). Third, although consecutive sampling with proportional allocation was employed to minimise bias, the dependence on routine records limited control over data quality. Fourth, during data collection, the medical records did not consistently capture information on health-system factors such as staff availability, PCR kit supply, or follow-up mechanisms and even lost data.

Strengths

Despite these limitations, the study has several important strengths. It is the first investigation in the Arsi Zone to specifically examine the timeliness of the initial PCR test among HIV-exposed infants, thereby filling a critical local evidence gap. The study included a large sample of 435 HIV-exposed infants drawn from three major hospitals, including one tertiary referral centre and accurately reflects current PMTCT performance in the zone. Consecutive sampling with proportional allocation across facilities enhanced representativeness and minimised selection bias. By focusing exclusively on the earliest stage of the EID cascade, the study provides highly actionable insights for program managers and policymakers to strengthen PMTCT services. These contributions are directly relevant to accelerating Ethiopia's progress toward the UNAIDS 95–95–95 targets and the global goal of eliminating pediatric HIV by 2030.

7. CONCLUSION AND RECOMMENDATIONS

Conclusion

Only 61.6% had a sample collected for the first PCR test 6 weeks of birth, which is markedly lower than the >95% global and national benchmark required to eliminate pediatric HIV by 2030. The timeliness of virological testing was longer than recommended, with extremely maternal age, being single or divorced, absence of antenatal care, mixed feeding, and home delivery identified as significant predictors of delayed testing. These patterns highlight gaps in maternal–child health services, particularly in early identification, counselling, and continuity of care for HIV-exposed infants. Overall, Timely EID in Arsi Zone remains unacceptably low, driven primarily by gaps in the maternal care pathway (home delivery, no ANC) and social vulnerability. Tackling these requires integrated community and health system interventions.

Recommendations

Ministry of Health (MOH)

- Revise national HEI guidelines to permit birth testing (PCR at 0–48 hours) for high-risk exposures, with clear cost-effectiveness criteria and implementation.
- Develop and roll out age-tailored PMTCT counselling materials for adolescent mothers (10–19 years) and women ≥35 years, and mandate integration of youth-friendly services in all facilities offering Option B+.

Arsi Zone Health Bureau

- Launch community health worker-led maternity waiting homes and transport voucher schemes in remote kebeles to increase institutional delivery rates by at least 20% within two years.
- Deploy health extension workers to conduct monthly home visits and phone/SMS reminders for ANC-4 completion and 6-week EID appointments.
- Partner with religious leaders and IDIR groups to deliver stigma-reduction sessions and escort HIV-positive mothers to facilities for delivery and infant testing.

Hospitals and Health Facilities

- Integrate DBS sample collection into labour/delivery wards, immediate postnatal care units, and EPI/immunization rooms, with mandatory checklists to prevent missed opportunities.
- Establish facility-based mother-to-mother peer support groups, targeting single, widowed, divorced, and adolescent mothers, with at least quarterly meetings.
- Ensure every ANC visit includes documented counselling on EID timing and return of results using standardised job aids.

Health Workers and Mother Support Group Members

- Provide individualized counselling and follow-up plans for adolescent mothers and women with parity ≥5, using pictorial tools to address stigma and time constraints.
- Actively promote and document maternal disclosure to at least one trusted family member or peer during ANC/postnatal visits.
- Implement active tracing (phone calls within 7 days and home visits within 14 days) for mothers who miss scheduled ANC, delivery, or EID appointments.

Researchers and Academic Institutions

- Conduct prospective cohort studies tracking HIV-exposed infants from birth to 18 months in Arsi Zone to quantify the long-term impact of maternal age, parity, disclosure, and residence on EID timeliness and retention in care.

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ANNEX 1.

Checklist to assess the timeliness of early infant hiv diagnosis and associated factors among hiv exposed infants in a public hospital in the Arsi zone, southeast, Ethiopia: 2025

No	Characteristic	Code	Skip
101	Age of the mother	_____in year	
102	Marital status of the mother	Single Married Window Divorced	
103	Residence of the mother	urban rural	
104	Place of delivery	at the Same facilities at Other facilities at Home	
105	If delivery at health facilities, NPV is received at birth	1. yes 2 . no	
106	Maternal education level	No formal education Primary Secondary Higher	
107	Occupation of the mother	Government occupation Daily laborer's Housewife Housekeeper	
108	Mother status known	Yes No	
109	If known, maternal HIV status is known at	1. At ANC 2. At delivery 3. At PNC 4. other	

110	If tested, the stage of HIV	One two Three Four	
111	Mother test for TB	Yes No	
112	If tested, the result	Positive Negative	
113	Mother tested for syphilis	yes no	
114	If tested, the result	positive negative	
115	infant feeding method of the mother	 EBF .ERE MF	
116	Sex of the infants	Male Female	
117	Weight of infant at birth	----------kg	
118	date of birth of the infants	_____days/month/year	
119	Date of sample collected for the first PCR test	----------day /months/year	
120	Is there a stockout reported	Yes No	
121	Outcome variable	Sample collected date-date of birth	
122	Sample collected for the first PCR test	Coded as 1 ~yes~ within 45 days(early diagnosis) Coded as 0~ no~ greater than 45 days (delay diagnosis	
123	If 1, the sample collected for	DBS GeneXpert	
124	The first PCR test result	Positive	

		Negative	
125	The first PCR test result received through	SMS Hard copy	
126	The presence of a mother support group at the facility	Yes No	
127	The overall positive results of the first PCR test		
128	POC for the first PCR test positive	-------days	
129	Turnaround time for the first PCR test	-------days	

ANEX 2:
Research Thesis Approval Sheet

Declaration of Advisors

We, the undersigned, hereby declare that we have carefully reviewed this research and provided guidance for its preparation. We confirm that this research is ready for defence with our approval as the university advisor(s), and to the best of our knowledge, the work presented meets the academic standards and is in partial fulfilment of the requirements for the degree of Master of Public Health in General Public Health.

1. Name of Principal Advisor: Mr Firaol Lemessa

Signature: __________

Date: _____

2. Name of Co-Advisor: Dr Abay Burusie

Signature: __________

Date: _____

Student Declaration

I, the undersigned, declare that I have incorporated the comments and suggestions of my advisors and examiners into the final version of my research.

Name of Student:

Hashim Gena :Signature: __________Date: _02/04/218 EC_____