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This research paper provides a review of the current state of early HIV diagnostic in children born to HIV infected mothers. It contains an analysis of international experience and provides arguments in favour of the introduction of modern methods, in particular, Dried Blood Spot (DBS) to ensure the earliest possible detection of HIV in the newborns and, correspondingly, to initiate antiretroviral therapy that would reduce HIV associated mortality among children. The research paper consists of four sections that sequentially cover all aspects of early HIV diagnostics and the estimated costs of introducing the DBS method into the routine national system for laboratory diagnostic of HIV infection. The research is intended for decision makers in the area of HIV/AIDS response, for the health administrators, heads of AIDS Prevention Centres and all other relevant specialists.

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1. Substantiation of Expediency of Early HIV Diagnostics in Children Born to HIV Infected Mothers

HIV diagnostics in adults and children, born to HIV negative mothers is performed by serological assays on the basis of detection of HIV antibodies in blood with the use of Enzyme Linked Immunosorbent Assay (ELISA) followed by a confirmatory test if positive (immunoblot (IB) or other ELISA test kit) [84, 87].

It is impossible to diagnose HIV with serological methods in infants born to HIV infected mothers. It is conditioned by the presence of maternal antibodies – immunoglobulines G (IgG) in the infant's bloodstream that are passively transferred to the foetus in utero through placenta. That is why in the first 9–18 months of life, positive test results of ELISA and WB tests can be obtained among all children born to HIV infected mothers, while only 2–31% of these children are actually infected [72, 84, 87]. The amount of time until the disappearance of maternal HIV antibodies from the infant's blood was studied in the African countries: the share of seronegative infants who were not HIV infected at the age of 9 months was 40%, in the age of 12 months – 93% and in the age of 18 months – 100% [47]. Analysis of the elimination of maternal HIV antibodies in 313 non-infected infants born to HIV infected mothers in Ukraine demonstrated the following share of seronegative children among those, who were actually not HIV infected: 44% at 12 months; 76% – at 15 months, 88% at 18 months and 99% at 21 months of age. The causes of later elimination of maternal antibodies were not studied, but perhaps it can be explained by the higher immunity stress among HIV infected women in Ukraine [75].

So, HIV the diagnosis of children born to HIV infected mothers can be made with the use of serological methods (based on the detection of HIV antibodies in bloodstream) only after the age of 18 months. Serologic assays (based on the negative HIV antibody test results) will help to exclude HIV positive diagnosis when they are 9–18 months of age [72, 84, and 87].

Early diagnosis of HIV infection in children born to HIV infected mothers is made with the use of virologic tests that detect genetic material or virus antibodies (strength of recommendations and quality of evidence guiding recommendations –

1A). The genetic material of HIV (proviral DNA or viral RNA) is identified with the polymerase chain reaction (PCR) assay. A qualitative detection of proviral DNA is performed in the white blood cells (mononuclear cells). Qualitative and quantitative detection of HIV RNA are performed in the blood plasma [19, 72, 84, and 87]. Virologic assays to diagnose HIV also include the ELISA method used to detect p24 HIV antigen in the blood serum or plasma. This assay is used in international practice much less frequently than PCR, because it provides much more non-specific (false positive or false negative) reactions among children in their first 4–6 weeks of life, which, on the one hand, is explained by the link of p24 HIV antigen of a HIV infected infant with the maternal antibodies with the development of immune complex, and on the other hand, by the cross-reaction of Ig-specific antibodies with the enzyme-marked antibodies and antibody solids of the ELISA test kit. Recently, an ultrasensitive p24 assay has been developed. It is based on thermal destruction of immune complexes and Ig-specific antibodies and it has been proven that this assay can be used for early diagnostic of HIV [72].

Early HIV diagnostic in perinatally infected children is expedient because without the timely initiation of antiretroviral (ARV) therapy 20–35% of HIV infected infants die before reaching the age of one year, and half of HIV infected infants may die by two years of age [40, 87]. The early use of ARV therapy protects the health and life to HIV infected children. It has been proven that the initiation of ARV therapy among perinatally HIV infected children in the age younger than three months significantly reduces the risk of HIV disease progression to AIDS, as well as mortality relating to immune deficiency [20, 32, and 83]. Infants, who started taking ARV drugs under three months old, had more stable and sustained reduction of viral load compared to children who began specific treatment after three months [1, 32, and 83]. Early HIV diagnostics and the early initiation of ARV therapy reduce infant mortality by 76%, reduce the frequency of conditions that develop due the progression of HIV infection and may result in the disability of a child (HIV encephalopathy, opportunistic infections), i.e., it helps to preserve life and health of HIV infected children [14, 83]. Early diagnosis of the infection improves medical follow-ups for HIV infected children and helps to save resources for the treatment of opportunistic infections [71].

The early exclusion of HIV gives psychological benefits to the family, reduces the risk of stigma and discrimination against the children and increases the chances for the orphaned children to be adopted. It also optimizes health management for non-infected children born to HIV infected mothers – that is, it provides an opportunity to discontinue cotrimoxasole prevention of pneumocystis pneumonia earlier, to vaccinate children for TB, which is very important in view of high prevalence of this disease among HIV infected adults [17]. Establishing the HIV status of children born to HIV infected mothers early on, provides an opportunity to quickly evaluate the efficiency of prevention of mother-to-child transmission (PMTCT) programmes and to strengthen control over their implementation [72, 84].

2. Sensitivity and Specificity of Laboratory HIV Diagnostic Assays

The diagnostic sensitivity (DS) of laboratory assays characterizes the probability of false positive test results if a person is infected. The diagnostic specificity (DSp) of a laboratory assay characterizes the probability of false negative test result in the absence of infection. To calculate DS and DSp, test results on the basis of a 'golden diagnostic standard' are interpreted as positive (A), false positive (B), false negative (C) and negative (D). DS is calculated using the formula:

$$DS = A : (A + C).$$

DSp is calculated using the formula:

$$DSp = D : (B + D)$$ [79].

When blood sample collection standards and testing techniques are observed, the number of false positive and false negative HIV test results depends on the sensitivity and specificity of test kits to the virus subtypes circulating in the researched population. The WHO recommends using virologic assays to diagnose HIV infection, which – provided the laboratory standards are observed – have a minimum sensitivity of 95% (ideally – 98%) and specificity of 98% (strength of recommendation and evidence guiding recommendations – 1B) [72, 83]. "Technical" causes of false positive results are explained by the high sensitivity of test kits and occur due to contamination of biological samples, most often on a pre-testing stage. False negative results are more frequently obtained due to the violation of storage and transportation conditions for biological samples [30, 59, 78, and 88].

An absolute number of false positive and false negative results are conditioned by the prevalence of HIV in the population examined. For instance, if the DS of a virologic assay is 95% and the DSp is 98% with 5% HIV prevalence among 10,000 children born to HIV infected mothers (500 children are HIV infected and 9,500 are non-infected), positive results will be detected in 665 cases (475 – HIV positive and 190 – false positive), and negative results – in 9,335 cases (9,310 – HIV negative, and 25 – false negative). In this case the prognostic value of the positive result at the first testing is 71.4%, and the prognostic value of the negative result is 99.7%. In conditions of 'one-time testing', 25 children with false-negative results are exposed to high risk of premature death caused by the delayed initiation of treatment, and 190 children with false positive results may start receiving unnecessary ARV therapy, which will affect their health and will imply economic losses. The second testing with the same test kit will help to establish HIV diagnosis with a high degree of reliability: the prognostic value of the second positive result is 99.2% and of the second negative result – 88.7% [72].

It should be noted that the lower the HIV prevalence is in the tested population, the lower the prognostic value of the first test results are. When the level of perinatal transmission of HIV goes down to 2%, the prognostic value of first positive test result for an infant born to HIV infected mother is reduced to 50.3% and the prognostic value of the second positive result equals 98% [72].

Thus, two positive virologic test results obtained from separate blood samples confirm HIV diagnosis irrespective of the child's age (strength of recommendations and evidence guiding recommendations – 1A).

3. Advantages and Disadvantages of Different Scenarios of Early HIV Diagnostics in Children

The first and foremost question of early HIV diagnostics is: when to test infants born to HIV infected mothers with virologic assays – on the first week of life, or between 4 to 6 weeks old?

The WHO strongly recommends conducting the first testing of infants born to HIV infected mothers as soon as possible. If possible, the first virologic testing should be conducted 48 hours after the birth. It is also recommended that all children born to HIV infected mothers are tested with virologic assays in the age of 4–6 weeks (strength of recommendations and evidence guiding recommendations – 1A) [71, 83, and 84].

It is well known that with formula feeding an infant can become HIV infected by his/her mother in utero or during delivery. In utero HIV infection can be confirmed by a positive result of the PCR test to detect HIV genetic material within the first 48 hours after birth. Negative test result during the first week of life and positive result after the first week of life indicate infection during delivery [42, 48]. In the absence of perinatal prevention of HIV transmission the probability of in utero infection is 27–35%, and probability of intrapartum infection reaches as much as 65% [23, 48, 56, and 66]. Studies conducted in the 1990-s demonstrated that in PCR testing of blood samples of infants born to HIV infected mothers to detect proviral HIV DNA in the first 48 hours after birth the diagnostic sensitivity of virologic assays was 38% (95% confidence interval (CI) – 29–46%). During the first week of life the DS is increasing insignificantly. On the second week of life the DS of this assay increases and at 14 days reaches 93% (95%, CI – 76–97%). In the age of 28 days the DS of assay for proviral DNA reaches 96% and DSp – 99% [65]. Further research in the USA demonstrated that in 1990–1992 when perinatal transmission f HIV was 18.1%, the level of in utero infection was 27%; in 1999 – 2000, after the introduction of comprehensive prevention of perinatal transmission of HIV, on the background of the reducing levels of HIV transmission up to 1.6%, the share of children who were infected in utero increased to 80% [56]. In Ukraine, the research conducted in 2002–2004 identified that among children in the first week of life the DS of the AmpliSens DNA-HIV assay of proviral DNA was 87% (95%, CI – 73–100%) [81].

So, the DS of virologic assays is lower than 95% when children are tested during the first week of life and becomes higher in the age of 4–6 weeks. However, children who were infected in utero urgently need early diagnostics and immediate initiation of ARV therapy. With the effort to prevent mother-to-child transmission, the share of children infected intrapartum is decreasing, while the share of children infected in utero is growing. This means that in case of successful implementation of PMTCT programmes, testing of infants for HIV in the first days of life (at the maternity hospitals) is becoming ever more informative.

It has been proven that in utero HIV infection is associated with a higher viral load and more severe natural progression of HIV infection. Such children develop HIV symptoms and severe immune deficiency earlier than children infected intrapartum, and may die earlier. In order to avoid adverse consequences and to reduce mortality associated with

severe immune deficiency all these children are in need of early start of ARV therapy. That is, children who were HIV infected in utero, are in need of early diagnostics in order to immediately initiate ARV therapy [23, 42, 53, 66, and 89]. In the USA the first testing for HIV is conducted among infants in their first two weeks of life [54].

Early initiation of ART among perinatally infected children born to HIV infected mothers, is considered so important that it recommended to immediately start ART for infants with a positive result of the first virologic assay (in particular, obtained 48 hours after birth), and simultaneously to take a new blood sample to confirm the positive result of the first test. Immediately initiating of ART saves the lives of HIV infected infants, that is why therapy cannot be delayed till the confirmation of the first positive virologic test result with the second positive result (strength of recommendations and evidence guiding recommendations – 1A) [32, 83]. It has been proven that informing parents physicians who perform medical follow-ups quickly about a positive result of the virologic test is conducive to early initiation of ART, and that is why this information shall be provided as soon as possible, but no later than four weeks after the blood sample collection (strength of recommendations and evidence guiding recommendations – 1A) [32, 83].

Another important question of early HIV diagnostics in children: what virologic assays should be used?

The WHO strongly recommends the use of the following virologic assays and corresponding samples of biological fluids for the examination: to detect HIV DNA in the whole blood or in the dried blood spot (DBS); to detect HIV RNA (qualitatively or quantitatively) in the blood plasma or in DBS; ultrasensitive assays to detect p24 antigen with dissociation of immune complexes in plasma or DBS (strength of recommendations and evidence guiding recommendations – 1A) [32, 72, 83, 84].

A qualitative detection of proviral HIV DNA is done by PCR assay in mononuclear blood cells. It is one of the most common and sensitive assays for early diagnostics of HIV infection in children who were prenatally exposed to HIV. This assay is reliable to detect HIV infection both in the absence of ARV prevention or ARV therapy for mother and/or her infant, and in case of perinatal effect of ARV medicines. This assay can detect HIV in the affected cells even in cases when viral load is undetectable due to ARV therapy received by a HIV infected patient. The existing test kits have rather high DS to detect different subtypes of HIV, in particular, B, C, D, E, G and H [46, 63, 65, and 88]. To the perform qualitative detection of proviral HIV DNA by PCR, a blood sample is taken from a vein of an infant after a fast and collected in the test tubes with 1/10 volume of anticoagulant – 3% EDTA solution. In order to avoid the destruction of cells, blood sample should be transported to laboratory at a temperature +2 … +8°C. Blood sample should be delivered to the laboratory within no more than 24–48 hours after blood was drawn [78].

HIV RNA is detected in the blood plasma and in DBS samples or in a blood plasma drop on the blotting paper using different testing methods. Most methods can detect viral load – the amount of HIV RNA in 1 mL of blood plasma; they are used to monitor HIV progression and to control the effectiveness of ARV therapy. There are following methods to detect HIV RNA: Real-time-PCR, B-DNA, transcription-mediated amplification

(TMA) and Nucleic acid sequence-based amplification (NASBA) [44, 72, 84, and 87]. A qualitative detection of HIV RNA by TMA assay and some other methods can be used as an alternative to early diagnostic of HIV infection [15, 84]. Testing methods for HIV RNA can detect a wide range of virus subtypes [2, 21, and 32]. Blood for HIV RNA testing is drawn in the morning after the night fast in the testing tube with 3% EDTA solution. In order to obtain plasma, the whole blood is centrifuged at 800–1600 g for 20 minutes. Plasma is then deep-frozen and stored frozen to perform the test [87].

In the 1990-s data was obtained showing that methods to detect HIV RNA in plasma during the first weeks of life demonstrated the same and even higher DS, compared to the methods to detect proviral HIV DNA [17, 18, 22, 45]. However, it is known that viral load on blood plasma is decreasing under the effect of ARV drugs and can become undetectable (the level falls lower than sensitivity level of a test kit, e.g., to less than 40 copies/mL). That is why there were some doubts about the reliability of the method to detect HIV RNA for early infant diagnostics on the background of parental ARV prevention of HIV transmission. Some studies have not found any significant decrease of DS of assays for testing HIV infected children who were on ARV drugs [44, 53, 55, and 64]. Still, it should be noted that in case when an infant was receiving ART at the moment of blood draw and had an undetectable viral load it does not exclude the probability of HIV infection [37].

Although HIV infected children, as a rule, have a very high viral load (significantly higher than adults) in the first months of life, detection of proviral DNA is used as first test for early infant diagnostics of HIV infection in order to avoid even the smallest probability of mistakes in relation to infants exposed to the prevention impact of ARV medicines. Methods for quantitative detection of HIV RNA should be used as a second, confirmatory virologic test. It will confirm the fact of HIV infection and also provide important information about the viral load in the blood plasma of HIV infected infant and will help to evaluate the risk of disease progression and risk of death [50, 70, 72, and 83].

When a HIV infected mother is breastfeeding, the infant can be infected at any time. That is why a positive result of virologic assay on the background of breastfeeding indicates HIV infection, while a negative result does not exclude the probability of infection. HIV diagnosis can be ruled out by virologic assays six weeks after discontinuation of breastfeeding [72, 84].

If the first result of a virologic test is positive and the second one is negative, there is a need to conduct the third test. Testing of a new specimen of the biological material with the same method can explain the reason of false result – be it contamination, violation of sample collection procedures, or transportation and storage of samples, or testing procedures. Secondary testing of the first and second specimens of biological material does not exclude the mistakes that has already occurred [72, 84, 87].

HIV diagnosis can be ruled out on the basis of two negative virologic test results (one of which was obtained for an infant in the age of older than two weeks, and the second one – after 4–6 weeks of life), or on the basis of two negative serologic tests in the age of 6–18 months, or one negative serologic test result in the age older than 18 months [54, 72, 84, 87].

4. Advantages and Disadvantages of the Use of DBS Technique for Early HIV Diagnostic in Children

Numerous studies have compared the DS and DSp of different assays used to detect HIV genetic material in whole blood samples and DBS. The studies demonstrated high DS and DSp of DBS assays and proved the option of using DBS for early HIV diagnostics in children [18, 46, 49, 57, and 68]. The detection of proviral DNA on DBS specimens by PCR method demonstrated the same levels of DS and DSp as on the whole blood samples [50]. A comparison of the assays to detect proviral HIV DNA and RNA on the DBS specimen confirmed their equally high DS (96–100%) and DSp levels (100%) [18]. A comparison of the PCR results on the whole blood samples(Amplicor DS 94%, Multiplex DS 100%, DSp 100%) and PCR on DBS specimen (S & S IsoCode DS 94%, Whatman DS 89,4%, DSp 100%) demonstrated similar high DS and DSp levels of these assays in infants aged 2–6 months. PCR testing of HIV RNA on blood plasma extracted from DBS (QL NASBA) demonstrated DS 89,7% and DSp 97,5% [68]. However, there is data to show that, if DBS is used for children with a low level of proviral DNA in blood, then false negative results can be obtained [7]. There is a potential probability of negative RNA HIV test result on DBS for HIV infected children born to HIV infected mothers, who were receiving prevention ARV regimens. Very often the low boundary of a quantitative detection of HIV RNA on DBS is 3,000–5,000 copies/mL [70, 72].

The DBS, or dried blood spot on filter paper, technique is used to detect viral load to monitor the effectiveness of ARV therapy and to identify HIV genotypic drug resistance [9, 12, 35, and 39]. The level of viral load in DBS differs insignificantly from the level found in the native blood plasma [24]. However, there are some limitations for the use of DBS to monitor viral load. Low level of biological material in the dried blood spot reduces the DS for the detection of HIV RNA with low viral load (<1,000–4,000 copies / mL) [5, 11, 41]. Analysis of the viral load on the whole blood samples collected on filter paper can give false positive results in people who receive efficient ARV therapy with an undetectable viral load due to contamination of proviral DNA from the white blood cells [25, 27]. Assays to detect HIV RNA from the whole blood samples on filter paper are less sensitive than from the plasma samples on filter paper, which is explained by the lower level of plasma in the whole blood sample. The number of viral RNA copies in the collected sample is proportional to its volume [73].

It has been established that the ultrasensitive p24 antigen assay can be used for early diagnostic of HIV infection from DBS. The sensitivity of this assay was 50% in the first days of life and increased to 80% during weeks 1–6 and to 95% – in the age older than six weeks of life. False negative results were found both among children, who were receiving ARV prevention, and among those, who were not receiving it. The DSp of this assay is 100% [28, 67].

5. Advantages and Disadvantages of the Use of DBS Technique for the Health Care Systems

The collection of a newborns' blood on filter paper – the dried blood spot technique (DBS) – has been used throughout the world for more than 40 years. For the first time this technique was offered by R. Guthrie in 1963 to screen the infants' blood for Phenylketonuria [33]. The DBS sample can be used to analyze a number of biochemical or genetic markers: amino acids, enzymes, hormones, genetic material (DNA, RNA), medicines, narcotic drugs etc. [69].

The method of DBS sample collection is recognized as a convenient form of screening to test a large number of individuals at the centralized laboratories located far from patients, when there is a need to transport samples [69]. DBS technique provides significant advantages for testing the newborns and infants, as the small amounts of blood are drawn for samples without the need of venipuncture. This method is less traumatic and is more acceptable for a mother. Maternity hospital personnel have a lot of experience collecting DBS samples and conducting the neonatal screening for Phenylketonuria and Hypothyreosis [11, 78].

The DPS sample collecting technique is simple and convenient, but it requires certain skills of the health personnel, a good supply of filter paper cards, the availability of special devices to dry the samples and expendable materials for their transportation. Blood for DBS sample is taken by heel or finger stick and then collected on a special filter paper – five spots (appx. 50 mcL) are usually prepared in accordance with the rules – a clearly visible spot on an each sheet of paper. Then paper with the samples is thoroughly dried at a room temperature for 4–12 hours. During the drying one should avoid the contact of the samples with other filter cards and to exclude exposure to sunlight and dust. Dried samples are separated from each other by a thin paper and then packed in the ziplock plastic bags with a special desiccant and humidity indicator. The package can be transported to a laboratory at room temperature within two weeks (it can be sent with a courier or by mail). Up to 10 samples can be placed in one bag (10 desiccants and one humidity indicator). The dried blood spot samples on filter paper can be stored at the temperature of +2 … +8° C during six weeks (possibly, even longer) [11, 69, 78].

The laboratory technicians conduct additional procedures to prepare the sample for analysis to extract the biological material from the filter paper for its testing, which requires special equipment (scissors or hand puncher, shaker) [11, 69, 78].

The possibility of drawing the blood sample by heel stick and the exclusion of venipuncture for infants reduces the risk of exposure of health care workers with HIV-containing biological fluids and reduces the risk of workplace emergencies. Unlike the transportation of test tubes with blood sample to the central laboratory, transportation of dried blood samples is safe and absolutely excludes the risk of emergency or exposure to the biological fluids during transportation [41].

Compared to the cases when proviral HIV DNA needs to be detected, transportation and storage of DBS samples does not require cold chain; there is no need for the urgent

delivery of DBS samples to the laboratory [41]. The conditions and the conditions for storing collected DBS samples for early HIV diagnostics, detection of viral load and genetic HIV drug resistance were also studied. Proviral DNA was stable in the DBS samples that were stored at room temperature during nine months. However, there is data to indicate that, after 1–3 months of storage at room temperature the viral load in samples was reducing [8]. At the same time, another multicenter study conducted in Northern America has identified the stable level of HIV RNA in the DBS samples stored at room temperature for at least one year [41]. Suboptimal storage of the DBS samples at the temperature of 37°C and air humidity of 100% led to a quick (within two weeks) deterioration of samples, due to which testing for genotypic drug resistance of the virus became impossible [53].

According to the experts [26, 43], the cost of DBS collections are less than USD 1 per one test, and transportation costs are much lower compared to the transportation of test tubes with blood or plasma. At the same time, the actual costs of lab tests remain the same, but analysis of a sample from DBS requires some additional time to extract the sample and biological material from it [26, 43].

The results of PCR testing from the DBS sample can be sent by mail, e-mail or by courier. Positive results can be immediately communicated by phone, which reduces the time to deliver this information. Taking into account the fact that test results are sent to a health care facility at the place of residence of an infant, the number of infants lost to medical follow-up is reduced [10, 35].

The use of DBS samples for analysis by PCR provides an opportunity to perform early diagnostics of HIV on the primary health care level, to decentralize not only the blood drawing, but also the provision of health care to children born to HIV infected mothers and expand access to early HIV diagnostics and treatment for such children. Correspondingly, a timely initiation of ARV for HIV infected children is the key to preserve their health and to reduce mortality of HIV infected children [9, 10, 14, and 58].

The convenience of blood drawing, the low volume of blood required for analysis (which does not require venipuncture in infants), the low cost of blood sample collection and transportation to the central laboratory, the decentralization of health care to children born to HIV infected mothers helped many countries with limited resources to standardize virologic testing for HIV from DBS. The introduction of this technique improved the coverage of children with the aim of early diagnostics and timely initiation of ARV therapy [9, 10, 14, 58, 72].

6. A Review of International Literature and Practice and Possible Ways to Introduce Early HIV Diagnostics in Children

An analysis of HIV genetic material by PCR assay for early infant diagnostics from the DBS samples compared to the analysis of the whole blood has a number of advantages with equal diagnostic sensitivity and specificity. The advantages are: convenience and safety of blood sample collection; low level of blood; venipuncture is not required to draw blood sample in infants; low cost of blood sample collection; easy and safe transportation of the samples to the central laboratory; cold chain is not required. Some disadvantages of the DBS analysis compared to the whole blood analysis include the need to have disposable materials; an additional stage is required to process the samples in the lab (additional equipment is required for this), and there is the need to train health personnel on the DBS sample collection technique.

The simplicity of DBS sample collection helps to conduct early HIV diagnostics on the primary health care level and at the maternity hospitals. That is why the introduction of the DBS technique is an important step to the decentralization of health care to children born to HIV infected mothers, it significantly scales-up access for children to early diagnostics of HIV infection and timely prescription of treatment, reduces the levels of disability and mortality among HIV infected children and reduces the costs related to it.

Together with the introduction of the DBS technique, there is a need to reform early HIV diagnostic in the country. There are several approaches to the development of early infant diagnostic.

Option 1. Procedures and terms of early diagnostic *remain unchanged* – AIDS centres collect DBS samples (first test – in the age of 1–2 months; in case of negative first test result the second test is performed in the age of 3–4 months; in case of a discordant result the third test is performed). The DBS samples are sent from the AIDS Centres to the laboratory to detect proviral HIV DNA by PCR assay. It is a "centralized" option, with which the DBS advantages include simplicity of blood drawing and convenience of transportation. A key disadvantage is that children still need to be taken to AIDS Centres.

Option 2. The terms of diagnostics remain the same, while DBS sample collection is delegated to the *primary health care level*, where blood samples are drawn, dried, packaged and then sent to the laboratory. The key advantages of this method are: a real "decentralization" of early diagnostics, "relief" for AIDS Centres from non-infected children; lack of risk of false positive results on a pre-testing stage. Key disadvantages include the possible delays with the supply of disposable materials; the need to send samples out one by one (because patients a 'geographically' dispersed); a lack of skills among health care workers of the primary health centres, etc.

Option 3. Children with perinatal HIV infection are first identified at the *maternity ward (maternity hospital)*: A blood sample is drawn from the heel of an infant for DBS on the second-fourth days of life; further early infant diagnostics is performed at the AIDS Centre in accordance with already introduced early diagnostic guidelines.

Such an option helps to identify children with high risk of mortality in the first months of their life and contributes to early initiation of ART. Pre-test counselling for mothers at a maternity hospital, and the motivation for her to come for the test results to the AIDS Centre will be an additional factor that would actually help to scale up the coverage of children born to HIV infected mothers by PCR diagnostics and timely initiation of ART. This option is preferable, because the health personnel of maternity hospitals already have the skills to collect DBS samples for neonatal screening for hereditary diseases and so the introduction of this technique will not cause any difficulties. A disadvantage of this approach is that 20% of HIV infected infants (who were infected intrapartum) will have actually negative result of the first test and positive result of the second test, and that is why a third test will be required. This option of the early HIV diagnostics in children with and additional examination in the age of 48 hours to detect antenatal HIV infection is included in the Clinical Guidelines on the Provision of Health Care to HIV Infected Children (approved at the forum of experts on March 19, 2013) and in the unified Clinical Guidelines on Health Care for HIV Diagnostics and Treatment among Children (*Fig. 1*).

7. Conclusions

1. According to the review of the specialized literature, the DBS method has been time proven and endorsed by many studies; it has similar sensitivity and specificity with the whole blood analysis, while remaining affordable to the patients and cost-efficient for the health care systems in comparison to the whole blood analysis, and so it can be recommended for a trial in a pilot study in Ukraine.

2. It will be expedient to introduction the DBS technique in Ukraine with simultaneous review of the guidelines for early HIV diagnostics in children and to conduct first testing in the maternity hospitals. Expected results of such change will include timely early diagnostics of HIV infection in children, who were infected antenatally, timely initiation of ART, reduced morbidity, disability and mortality among HIV infected children, and reduced costs related to it.

3. In case of successful results of the DBS technique trial the next step may include a gradual decentralization of blood drawing through the involvement of mobile labs that exist at some AIDS Centres, infectious disease centres at the place of residence of HIV infected infants in the process of the DBS sample collection, which will really make early diagnostics of HIV infection closer to the patients and increase their coverage with PCR testing.

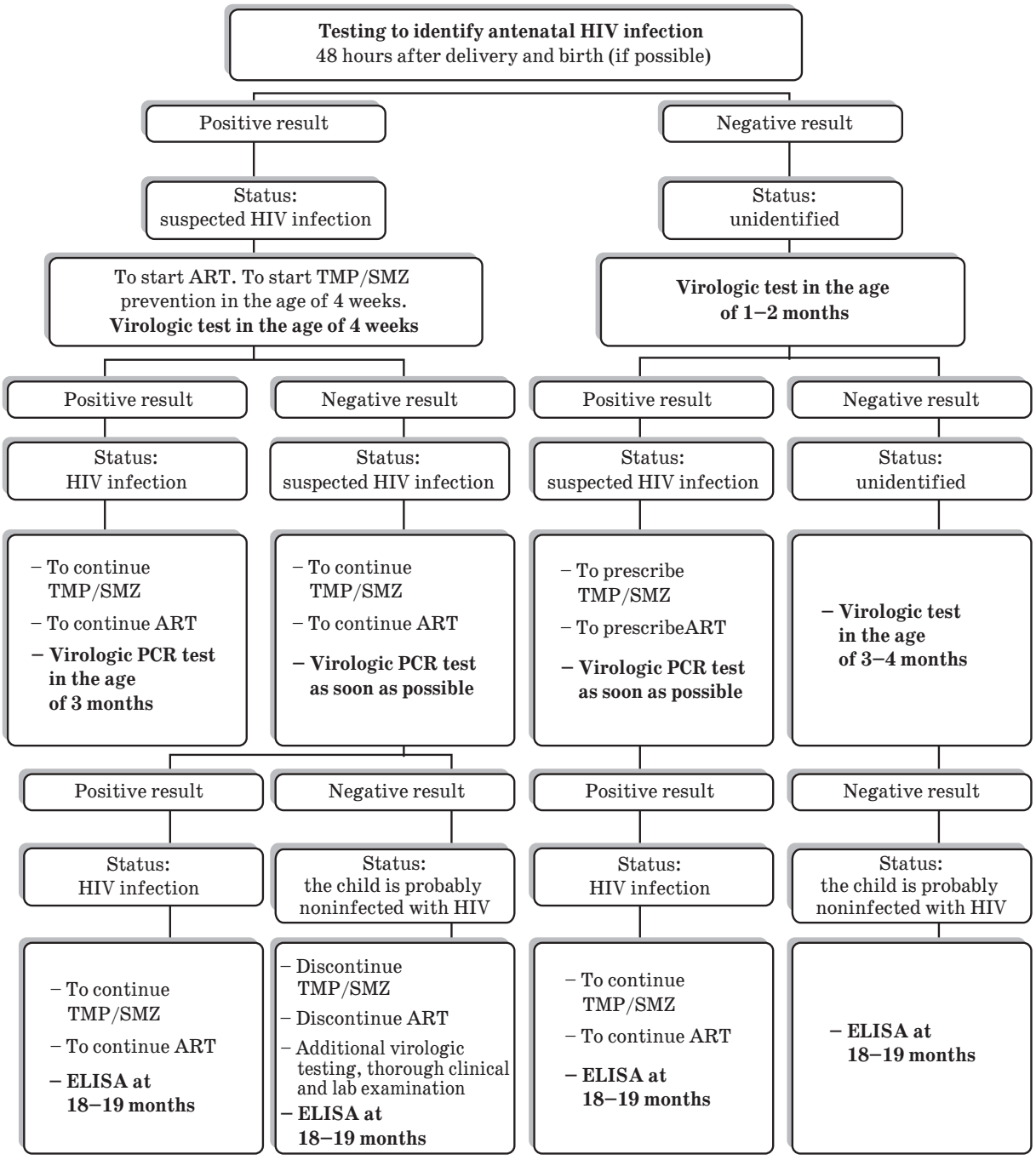

Fig. 1. **Guidelines for early HIV diagnostics in children born to HIV infected mothers on a formula feeding**

II. A Review of the Existing System for Early Detection of HIV among Newborns in Ukraine. Challenges and Gaps in the Early Diagnostics System

N. O. Babiy, I. V. Andrianova

According to the Ukrainian Centre of Control for Socially Dangerous Diseases at the Ministry of Health of Ukraine, the share of women among HIV infected people in Ukraine is permanently growing and reached almost 40.5% in 2012. The growth of heterosexual transmission and number of HIV infected women of childbearing age resulted in the increasing numbers of children born to HIV infected mothers: in recent years more than 4,000 births by HIV infected mothers have been reported annually. In 2010, HIV infected mothers gave birth to 4,049 children, in 2011 – 4,010 children and in 2012 – 4,048 children. And though HIV infection rates among pregnant women have a declining trend in the recent years (in 2009 – by 0.33%, in 2010 – by 0.28%, in 2011 – by 0.26% and in 2012 – by 0.24%), the absolute number of pregnant women with newly detected HIV infection remains on the level of 2,500 people in the last 3 years. Some of these women became HIV infected in the second half of their pregnancy, which, as a rule, results in the high level of mother-to-child transmission of HIV. This statement is also relevant in the absence of any prevention interventions during pregnancy and delivery (the risk to give birth to HIV infected child is 25% to 40%) [3, 6]. At the same time, the introduction of some evidence-based prevention interventions (specific chemoprophylaxis with ARV medicines, selection of the optimal delivery method, and avoidance of breastfeeding) may help to reduce the risk of vertical transmission to less than 2% [4].

According to experts from the Ministry of Health of Ukraine, prevention of mother-to-child transmission of HIV remains the only prevention programme today that ensures a sufficient coverage of the target population in the country. The practice of testing every pregnant woman (who decided to preserve her pregnancy) twice during pregnancy for antibodies to HIV-1/2 helped to identify 2,696 HIV infected women in 2012 (including 63 women, who were infected in the second half of pregnancy) and to take necessary prevention measures.

The efficient implementation of the programme to prevent vertical transmission of HIV from mother to child in Ukraine resulted in a significant reduction of frequency of mother-to-child transmission – from 27.8% in 2001 to 6.3% in 2008 and to 4.94% (177 children) in 2010 (*Fig. 2*) [76].

However, a lot still needs to be done to get the number of cases of mother-to-child transmission of HIV down to zero, as defined in the National Targeted Social Programme in Response to HIV /AIDS for 2014–2018.

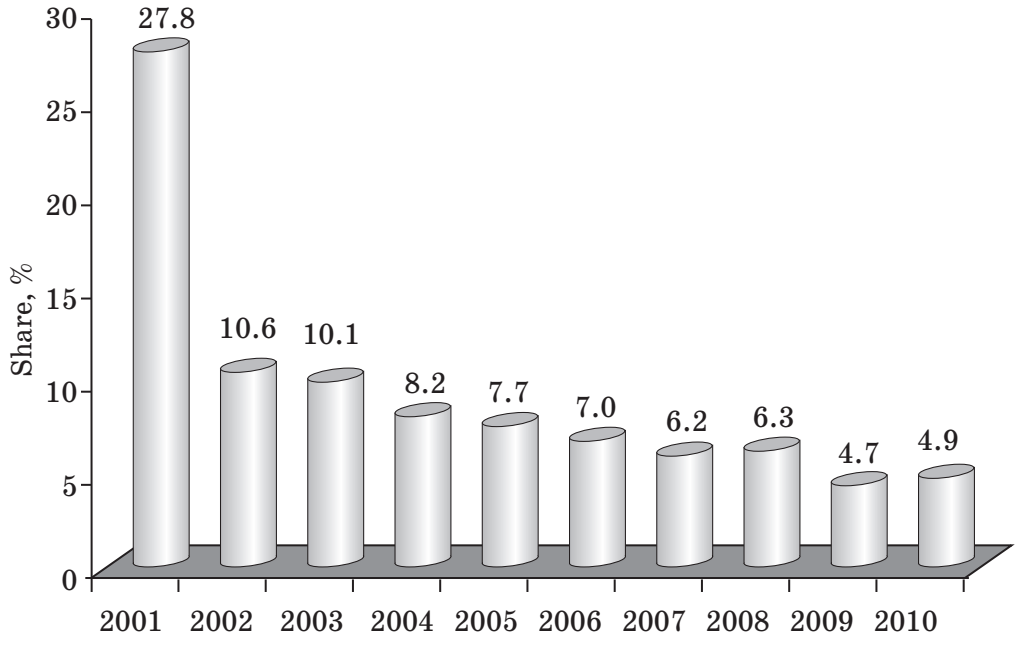

Fig. 2. **Dynamics of vertical transmission of HIV in Ukraine**

It is known that the use of ELISA assay to identify the status of an infant during the first 18 months of life is not expedient because maternal antibodies still circulate in the infant's bloodstream. Currently the best method to diagnose HIV infection in children younger than 18 months of age is detection of virus or its components in the blood samples, i.e., detection of proviral HIV-1 DNA or RNA by polymerase chain reaction (PCR) assay and ultrasensitive test for p24 antigen. The use of techniques to detect HIV-1 RNA has not become widespread because this method is expensive in comparison to others and it requires using the blood plasma, which is rather difficult to take from infants. Ultrasensitive assay to detect p24 antigen was earlier considered less sensitive than PCR assays [31], although a new, modified version of this test has the same sensitivity and specificity, as PCR tests [62]. Still, it is rather labour-intensive to perform. That is why the technique to detect proviral DNA in the blood samples has become the most common method in the global laboratory practice for the purposes of early HIV diagnostics in children born to HIV infected mothers. Sensitivity of this technique increases with the age of an infant: 48 hours after birth it is 38%, and on the 28th day after birth – 98% [13, 60].

Since the end of 2005 in Ukraine, the HIV status of children born to HIV infected mothers during first 18 months of life was established by testing blood samples for proviral HIV-1 DNA by PCR technique. Slightly later, in 2007, on the basis of then valid WHO guidelines and taking into account the economic situation in the country, the Ministry of Health of Ukraine developed and approved the order #740 of 23 November 2007 that specified the guidelines for early HIV diagnostics in infants younger than 18 months of age. These guidelines are still valid. According to the algorithm that is specified there, testing of blood samples of infants born to HIV infected mothers for proviral DNA should be conducted twice: first test in the age of one-two months and the repeated test – in the age of 3–4 months, if the first test

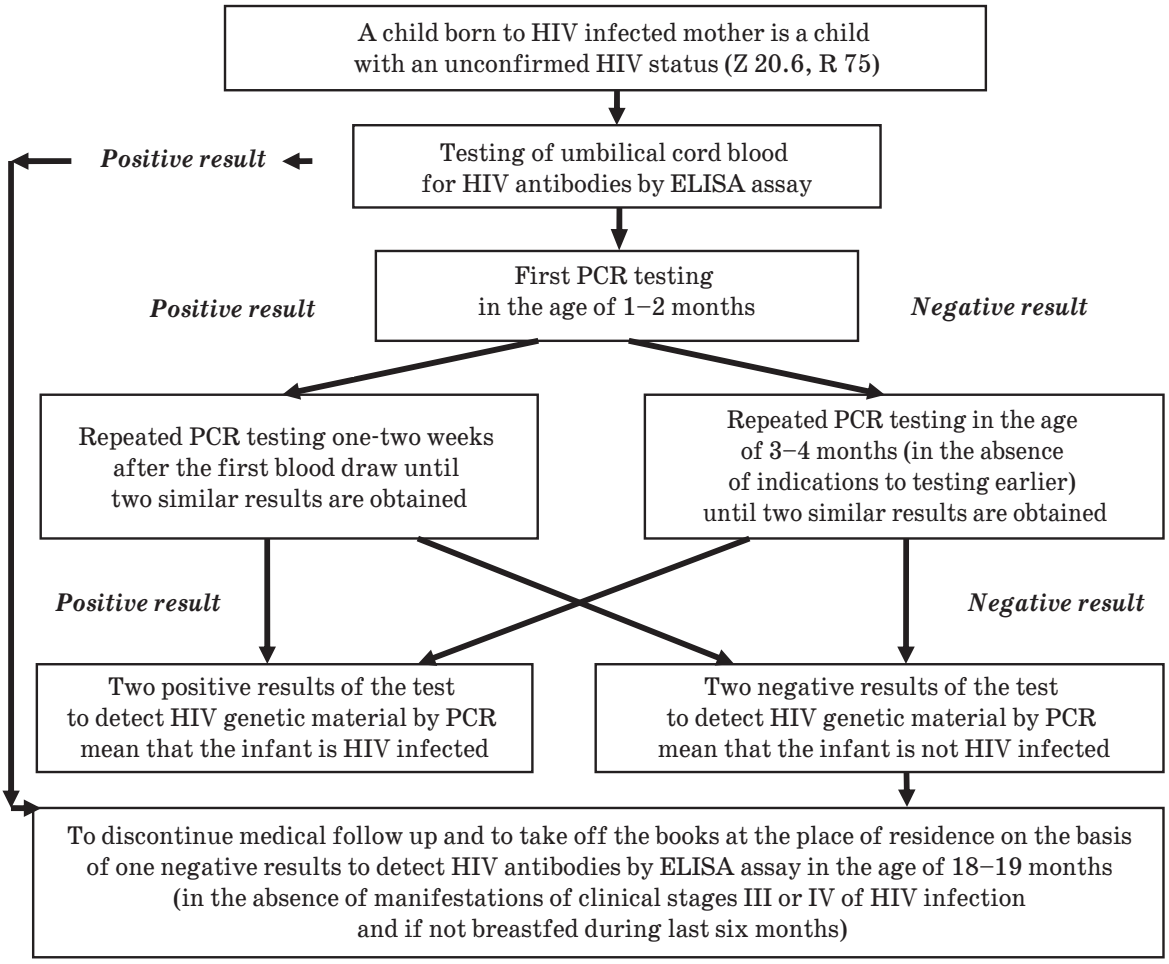

Fig. 3. **Algorithm for early diagnostics and exclusion of HIV diagnosis in children born to HIV infected mothers (Order of the MoH of Ukraine # 740 as of 23 November 2007)**

result was negative. If the first result was positive, it is recommended to perform a repeated test in 1–2 weeks 1–2 (*Fig. 3*). In order to establish the final diagnosis, the blood serum samples are tested for antibodies to HIV by ELISA and Immune Blot methods after the child reaches the age of 18 months.

HIV diagnosis for an infant, who was not perinatally exposed to HIV but has clinical signs, laboratory confirmed immune suppression and epidemiological indications should be made by detecting HIV antibodies in the sample of venous blood, on the basis of positive ELISA test result that is to be confirmed by Immune Blot assay.

In 2005–2006 testing of infant blood samples by PCR for HIV-1 DNA was performed only at the laboratory of Ukrainian AIDS Prevention Centre where all the blood samples of infants born to HIV infected mothers from all regions of Ukraine were sent.

It is also known that, at that period, there was a rather serious problem with transportation of infant blood samples from other, especially distant, regions of Ukraine. Besides, the blood draw for this test in most regions was only performed in the medical manipulation rooms at regional (oblast) and sometimes city AIDS Centres, which,

in its turn, made children and their parents from distant districts to go to the oblast AIDS Centres. The reluctance of parents to bring a child to the examination, a complete refusal from it, lack of money to pay for the transport, lack of permanent place of residence – it is just an incomplete list of problems that explained the low coverage of children with testing in the first years of introduction of this technique in Ukraine. For example, in 2006 not a single blood sample of children born to HIV infected mothers in AR Crimea and Ivano-Frankivsk oblasts was sent to the laboratory of Ukrainian AIDS Centre to be tested for proviral HIV-1 DNA.

It should be noted that one of the key reasons for the poor coverage of children with testing in other countries, such as the Russian Federation, was the refusal of parents to treat their children and care for them. According to the Ministry of Health of Russian Federation, around 12% of children born to HIV infected mothers are not available for follow up, 5–8% parents refuse to examine children for HIV diagnostics, 7% refuse from testing and follow-up for their children, 4% refuse to treat their HIV infected children [77].

Besides, in the first couple of years after the introduction of this technique in Ukraine the quality of blood samples sent for analysis was rather low due to the violation of rules and requirements of the pre-testing stage: blood was drawn with the test tubes with heparin, which is unacceptable for PCR assay; blood was drawn not with the special vacuum systems, such as Venoject (with EDTA) or Vacuett ® (with K3-EDTA or K2-EDTA), but with syringes, and the storage temperature regime was not observed during transportation, etc.

Starting from 2007, pursuant to the Order of the Ministry of Health of Ukraine #516 as of 25 July 2006, early HIV diagnostics tests for children born to HIV infected mothers were introduced, in addition to Ukrainian AIDS Prevention Centre, in Odessa Oblast and Crimean Republican AIDS Prevention Centres. The Laboratory of Odessa Oblast AIDS Prevention Centre was designated as interregional laboratory for Mykolayiv and Kirovograd oblasts, and laboratory of Crimean Republican AIDS Prevention Centre – for Sevastopol city and Kherson oblast. Other regional AIDS Centres (in 20 oblasts and Kyiv city) were served by the laboratory of Ukrainian AIDS Prevention Centre.

In the first months after the introduction of PCR technique in the laboratory of Odessa AIDS Prevention Centre for early HIV diagnostics in children born to HIV infected mothers, the system of blood delivery for early diagnostic tests from the districts of Odessa oblasts, from Odessa city and attached oblasts (Mykolayiv and Kirovograd) was not very well coordinated. Because of this, the laboratory technicians did not have enough time to use diagnostic kits before the expiration of their shelf life. This is why three out of every 28 test kits for the detection of proviral HIV-1 DNA allocated to Odessa AIDS Centre were returned to Ukrainian AIDS Prevention Centre. However, according to the specialists from the Odessa AIDS Centre, the share of children covered with this testing was rather high: in 2007, of 374 children born to HIV positive mothers in Odessa oblast 349 infants (93% of the total number) were tested by PCR technique once and 186 infants (49%) – twice. In Mykolayiv oblast the share of children covered with testing in 2007 was 96.1% and only in Kirovograd oblast this indicator was lower – 66.7%.

Data on the activities of interregional laboratory based on the Crimean Republican AIDS Prevention Centre were rather limited, but according to the information provided by the laboratory technicians, the coverage level was above 90%.

In 2005–2007 the laboratory of Ukrainian AIDS Prevention Centre tested 3,050 children for HIV DNA. Positive test results were established for 314 children, which means that the level of vertical transmission of HIV was 10.3 ± 0.5%. It should be noted that on the basis of ELISA tests this indicator was lower and equalled to 7.7% in 2005 and 6.2% in 2008 (*Fig. 2*).

Due to the annual growth in the number of children born to HIV infected mothers that were observed in Ukraine, the burden of interregional laboratories has significantly increased. In 2008–2009 Ukrainian AIDS Prevention Centre in some days received more than 200 blood samples of children younger than 18 months to be tested for proviral DNA. It is known that this kind of analysis is done on whole blood, which can be used for the test only within 48 hours from the moment of drawing, which limits the time for blood sample testing to 24 hours. Excessive workload and impossibility to process such number of samples during one working day (due to limited time and technical capacity of equipment used for this test) made the laboratory technicians introduce the schedule of acceptance of clinical specimens with the exact specification of their number and in proportion to the needs of each region in this type of tests. However, the requirement to meet the schedule created additional challenges for the paediatricians related to the organization of the process of biological material collection and delivery to the laboratory. There were problems in some regions with the observance of schedule for blood drawing. Limited funds for business trips also complicated the delivery of blood samples from regional AIDS Centres (e.g., Donetsk and Kharkiv AIDS Centres) to interregional laboratories in accordance with the suggested schedule.

All these challenges required an urgent solution. In 2009 they managed to ensure the delivery of equipment needed to conduct tests for proviral HIV-1 DNA to several other regional AIDS Centres and so, in 2009 it became possible to further decentralize this type of tests.

According to the Order of the Ministry of Health of Ukraine # 673 as of 16 September 2009, six interregional laboratories were designated to conduct early HIV diagnostic assays among children younger than 18 months of age (*Table 1*).

In order to implement the requirements of this order, and to learn the technique required for the detection of proviral HIV-1 DNA in the blood samples, the laboratory of the Ukrainian AIDS Prevention Centre was conducting training and internship courses for the specialists from the regional AIDS Centres. In 2009 specialists from Dnipropetrovsk Oblast and Kyiv City AIDS Centres participated in the training workshops in the laboratory of Ukrainian AIDS Centre.

In general, according to the reports on the number and results of tests to detect proviral HIV-1 DNA submitted to the Ukrainian AIDS Prevention Centre at the Ministry of Health of Ukraine, 8,904 such tests were conducted in 2009, of which 5,216 tests were performed directly by the specialists of this facility. According to their data the level vertical transmission of HIV in this period was 5.61 ± 0.96%. Later on, when children

Table 1

List of AIDS Prevention Centres to Conduct Laboratory Monitoring of HIV Infection and Antiretroviral Therapy with the List of Health Care Facilities in the Oblasts, Which Will Conduct Analyses (Virologic Assays to Identify Proviral HIV-1 DNA)

##	Location of interregional laboratories	List of oblasts
1	Crimean Republican AIDS Prevention Centre	AR Crimea, Kherson oblast, Sevastopol city
2	Ivano-Frankivsk Oblast AIDS Prevention Centre	Ivano-Frankivsk, Volyn, Zakarpattia, Lviv, Rivne, Chernivtsy, Ternopil, Khmelnytsky oblasts
3	Dnipropetrovsk Oblast AIDS Prevention Centre	Dnipropetrovsk oblast
4	Odessa Oblast AIDS Prevention Centre	Odessa, Kirovograd, Mykolayiv oblasts
5	Kyiv City AIDS Prevention Centre	Vinnytsa, Cherkasy, Zhytomyr oblasts, Kyiv city
6	Ukrainian AIDS Prevention Centre	Kyiv, Poltava, Sumy, Kharkiv, Chernigiv, Donetsk, Lugansk, Zaporizhzhya oblasts

Table 2

List of AIDS Prevention Centres to Conduct Laboratory Monitoring of HIV Infection and Antiretroviral Therapy with the List of Health Care Facilities in the Oblasts, Which Will Conduct Analyses (Virologic Assays to Identify Proviral HIV-1 DNA)

##	Location of interregional laboratories	List of oblasts
1	Crimean Republican AIDS Prevention Centre	AR Crimea, Kherson oblast, Sevastopol city
2	Dnipropetrovsk Oblast AIDS Prevention Centre	Dnipropetrovsk oblast
3	Ivano-Frankivsk Oblast AIDS Prevention Centre	Ivano-Frankivsk, Volyn, Zakarpattia, Lviv, Rivne, Chernivtsy, Ternopil, Khmelnytsky oblasts
4	Odessa Oblast AIDS Prevention Centre	Odessa, Kirovograd, Mykolayiv oblasts
5	Poltava Oblast AIDS Prevention Centre	Poltava oblast
6	Kyiv City AIDS Prevention Centre	Vinnytsa, Cherkasy, Zhytomyr oblasts, Kyiv city
7	Ukrainian AIDS Prevention Centre	Kyiv, Sumy, Kharkiv, Chernigiv, Donetsk, Lugansk, Zaporizhzhya oblasts

who reached the age of 18 months were tested for HIV antibodies by ELISA and Immune Blot assays, this indicator decreased to 4.7% (*Fig. 2*).

According to an Order from the Ministry of Health of Ukraine # 673 as of 16 September 2009 (in the version of the Order #469 of the MoH of Ukraine as of 8 June 2010), seven interregional laboratories were designated to conduct early HIV diagnostic assays among children younger than 18 months of age starting from April 2010 (*Table 2*).

In order to improve the procedures for data collection about the number of children born to HIV Infected mothers and their coverage with testing to detect proviral HIV-1 DNA, specialists from the Reference Laboratory of the Ukrainian AIDS Prevention Centre developed new recording and reporting forms and introduced them in 2011. Information that was collected helped to learn the number of tests among children born within a year preceding the reporting year, as well as the number of children, who had not yet reached the age of two months and did not need testing within the reporting year (*Table 3*).

Although the Ukrainian Centre of Control for Socially Dangerous Diseases at the Ministry of Health of Ukraine received data about the number of children born in 2011 and tested in 2012 not from all regions of Ukraine, it can be seen in *Table 6* that total of 4,291 children were tested in 2012, which is more than the number of children born in 2012. This discrepancy is caused by the fact that in 2012 they tested children born both in 2011 and in the current year. At the same time, of 4,048 children born in 2012 only 3,245 children were tested, which is 80.2% of the total number. Most of them (2,397) were tested twice because by the end of the year they reached the age of 3–4 months, and 826 children were tested only once. Another 803 children born in 2012 should be tested in 2013. Due to the discordant (13) and dubious (9) results of the tests, 22 children were tested 3 times, which is 0.68% of all tested children.

Data about coverage of children with testing to detect proviral HIV-1 DNA in the regions in 2012 is provided in *Table 4*.

As seen in the table, the coverage of testing in different regions varied from 47.2% (in Zhytomyr oblast) to 144.0% (in Chernivtsy oblast).

Most frequently, the reasons of under testing of children include:

- A violation of the system for the uninterrupted supply of test kits and disposable materials to oblast laboratories, which is explained by regular stock outs in the centralized procurement of these materials for the state budget funds.
- Late enrolment of children born to HIV infected mothers into care.
- The refusal of parents to test their children, etc.

However, it should be noted that the data provided in *Table 4* is tentative; taking into account the lack of the uniform computer database of HIV infected people in the country and the lack of complete and objective information about the number and frequency of tests among children.

Table 3

The Number of blood samples tested in 2012 for proviral HIV-1 DNA for the purpose of early diagnostics of HIV in children born to HIV infected mothers

##	Region (oblast) of Ukraine	Total number of children tested in 2012	Children born in 2011, tested				Children born in 2012, tested				
			Total	Once	Twice	Positive results	Total	Once	Twice	Three times	Positive results
1	AR Crimea	263	77	33	44	1	186	45	139	2	5
2	Vinnytsa	48	0	0	0	0	48	17	31	0	2
3	Volyn	32	2	2	0	0	30	23	7	0	0
4	Dnipropetrovsk	592	144	162	9	0	448	137	305	6	26
5	Donetsk	772	219	84	135	8	553	134	414	5	24
6	Zhytomyr	51	0	0	0	0	51	21	30	0	0
7	Zakarpattia	20	9	6	3	0	11	3	8	0	0
8	Zaporizhzhya	128	42	36	6	0	86	20	66	0	2
9	Ivano-Frankivsk	25	0	0	0	0	25	3	22	0	0
10	Kyiv	174	29	3	26	1	145	37	108	0	3
11	Kirovograd	121	13	1	12	2	108	8	99	1	4
12	Lugansk	176	50	28	22	3	126	30	96	0	3
13	Lviv	46	5	0	5	0	41	19	22	0	0
14	Mykolayiv	278	81	41	40	1	197	27	170	0	9
15	Odessa	480	104	55	49	5	376	66	310	0	19
16	Poltava	58	9	8	1	0	49	36	13	0	1
17	Rivne	68	27	21	6	0	41	7	27	7	2
18	Sumy	34	0	0	0	0	34	2	32	0	0
19	Ternopil	11	0	0	0	0	11	1	10	0	0
20	Kharkiv	112	38	16	22	1	74	30	44	0	0
21	Kherson	150	41	23	18	0	109	13	96	0	1
22	Khmelnytsky	87	37	24	13	1	50	15	35	0	1
23	Cherkasy	123	35	22	13	0	88	22	66	0	2
24	Chernivtsy	45	9	6	2	0	36	18	18	0	0
25	Chernigiv	112	34	21	13	2	78	14	63	1	2
26	Kyiv city	245	40	3	37	1	205	48	157	0	2
27	Sevastopol city	40	1	1	0	0	39	30	9	0	1
	Total	**4291**	**1046**	**596**	**476**	**26**	**3245**	**826**	**2397**	**22**	**109**

Table 4

The Number of children tested for proviral HIV-1 DNA For the purpose of early HIV diagnostics in 2012

##	Region (oblast)	Number of children born	Number of tested children	Coverage, %
1	AR Crimea	222	186	83.8
2	Vinnytsa	63	48	76.2
3	Volyn	53	30	56.6
4	Dnipropetrovsk	558	448	80.3
5	Donetsk	722	553	76.6
6	Zhytomyr	108	51	47.2
7	Zakarpattia	18	11	61.1
8	Zaporizhzhya	104	86	82.7
9	Ivano-Frankivsk	32	25	78.1
10	Kyiv	163	145	89.0
11	Kirovograd	106	108	101.9
12	Lugansk	149	126	84.6
13	Lviv	84	41	48.8
14	Mykolayiv	241	197	81.7
15	Odessa	469	376	80.2
16	Poltava	72	49	68.1
17	Rivne	50	41	82.0
18	Sumy	46	34	73.9
19	Ternopil	13	11	84.6
20	Kharkiv	104	74	71.2
21	Kherson	105	109	103.8
22	Khmelnytsky	64	50	78.1
23	Cherkasy	103	88	85.4
24	Chernivtsy	25	36	144.0
25	Chernigiv	90	78	86.7
26	Kyiv city	242	205	84.7
27	Sevastopol city	42	39	92.9
	Total	4048	3245	80.2

The data can also indicate that it is practically impossible to define the level of coverage with testing of children born to HIV infected mothers, as well as the needs of each region in testing to detect proviral HIV-1 DNA. The situation becomes even more complicated by the fact that the share of children born in the previous year (as a rule, those who were born in autumn and winter) require a repeated test in the current year; besides, children who do not reach the age of one month before the end of the year should have two tests in the current year. At the same time, the calculated needs should also include reagents needed for additional analyses in case of discordant or dubious test results. When the need for test kit procurement is calculated, the experts have the data on birth rate only for the previous year, but they do not take into account additional number of children born in the end of previous year, who should be tested, as well as the number of children who will not have a chance to be tested during the current year. That is why there is a need to develop and align the method to assess needs taking into account all the above factors.

In 2013 the purchase of reagents and disposable materials has been planned to test 5,430 children (*Table 5*) from the state budget, which is by 31.1% more compared to 2012.

According to preliminary data, the results of tests that have already been conducted and diagnosed, the level of vertical transmission may be 3.36% in 2012, which is lower than in 2009. In some regions this indicator differs from the average one (*Table 6*). However, the final conclusions about the rate of vertical transmission can be made only after collection of data about testing of all children born in 2012 and after conducting ELISA and Immune Blot assays when these children reach the age of 18 months.

It should be noted that today, the quality of clinical samples sent to interregional laboratories from oblast and city AIDS Centres is much higher compared to c 2005–2006 when this testing techniques was just being introduced in Ukraine; however, even today a certain portion of blood samples are not taken for analysis because of their low quality. For example, in 2012 of 2,580 blood samples sent to the laboratory of Ukrainian AIDS Centre 87 (3.4%) samples were rejected (blood clots, haemolysis, and insufficient volume of material) (*Table 7*).

Monitoring visits to the manipulation rooms in some regional AIDS Centres revealed that blood drawing procedures for infants were sometimes violated: nurses used syringes instead of vacuum systems, motivating it by difficulties to perform venipuncture and draw venous blood from the newborns; after drawing the blood into syringe they open the cap of a test tube with anticoagulant and transfer blood there, which is a violation of sterility requirements for PCR assays; the sampled blood is then stored at room temperature; sometimes the clinical samples are wrongly marked. As soon as blood is not thoroughly mixed with anticoagulant, the fibrin clots are formed in the sample which made them unacceptable for testing. As the long term practice shows, the presence of fibrin clots is one of the key reasons to reject the blood samples sent for PCR testing to detect proviral HIV-1 DNA. Violation of the storage and transportation temperature regime for blood samples, direct contact of test tubes containing biological materials with cooling elements leads to blood haemolysis and, consequently, to the rejection of these samples from testing.

Table 5

Number of blood samples that can be tested to detect proviral HIV-1 DNA in 2013

##	Location of interregional laboratory	The list of served regional AIDS Centres	Approximate number of blood samples that can be tested during a year (till 8 November 2013)
1	Crimean Republican AIDS Prevention Centre	Crimean Republican	446
		Kherson Oblast	254
		Sevastopol City AIDS Centre	68
		Total	768
2	Dnipropetrovsk Oblast AIDS Prevention Centre	Dnipropetrovsk Oblast	1305
		Total	1305
3	Ivano-Frankivsk Oblast AIDS Prevention Centre	Ivano-Frankivsk Oblast	58
		Volyn Oblast	74
		Zakarpattia Oblast	46
		Lviv Oblast	106
		Rivne Oblast	156
		Chernivtsy Oblast	103
		Ternopil Oblast	25
		Khmelnytsky Oblast	200
		Total	768
4	Odessa Oblast AIDS Prevention Centre	Odessa Oblast	587
		Kirovograd Oblast	148
		Mykolayiv Oblast	340
		Total	1075
5	Poltava Oblast AIDS Prevention Centre	Poltava Oblast	
		Total	80
6	Kyiv City AIDS Prevention Centre	Vinnytsa Oblast	87
		Zhytomyr Oblast	92
		Cherkasy Oblast	223
		Kyiv city	443
		Total	845
7	Ukrainian AIDS Prevention Centre	Donetsk Oblast	1394
		Zaporizhzhya Oblast	231
		Kyiv Oblast	314
		Lugansk Oblast	318
		Sumy Oblast	62
		Kharkiv Oblast	202
		Chernigiv Oblast	202
		NCSH “OKHMATDYT”	41
		Total	2764

Table 6

The number of children in Ukraine who had early HIV diagnostics by PCR assay in 2012

##	Regions from which samples were sent	Number of tested children	Number of children in the blood of how HIV-1 DNA was detected	
			Absolute	Relative, %
1	AR Crimea	186	5	2.69
2	Vinnytsa	48	2	4.17
3	Volyn	30	0	0
4	Dnipropetrovsk	448	26	5.80
5	Donetsk	553	24	4.34
6	Zhytomyr	51	0	0
7	Zakarpattia	11	0	0
8	Zaporizhzhya	86	2	2.33
9	Ivano-Frankivsk	25	0	0
10	Kyiv	145	3	2.07
11	Kirovograd	108	4	3.70
12	Lugansk	126	3	2.38
13	Lviv	41	0	0
14	Mykolayiv	197	9	4.57
15	Odessa	376	19	5.05
16	Poltava	49	1	2.04
17	Rivne	41	2	4.88
18	Sumy	34	0	0
19	Ternopil	11	0	0
20	Kharkiv	74	0	0
21	Kherson	109	1	0.92
22	Khmelnytsky	50	1	2.00
23	Cherkasy	88	2	2.27
24	Chernivtsy	36	0	0
25	Chernigiv	78	2	2.56
26	Kyiv city	205	2	0.95
27	Sevastopol city	39	1	2.56
	Total	3245	109	3.36

Table 7

Frequency of rejection of blood samples
For the tests to detect proviral HIV-1 DNA
in the laboratory of Ukrainian AIDS Centre (in 2012)

##	Regions from which samples were sent	Number of blood samples sent for testing	Number of rejected blood samples	
			Absolute	Relative, %
1	Bila Tserkva, city	69	2	2.9
2	Donetsk Oblast	1236	41	3.3
3	Zaporizhzhya Oblast	229	5	2.2
4	Kyiv Oblast	276	23	8.3
5	Lugansk Oblast	298	6	2
6	Sumy Oblast	87	1	1.1
7	Kharkiv Oblast	179	5	2.8
8	Chernigiv Oblast	183	3	1.6
9	NCSH "OKHMATDYT"	23	1	4.3
	Total	2580	87	3.4

Evaluation of the situation with early HIV diagnostics in children born to HIV infected mothers in Ukraine has revealed the following problems that complicate testing with high efficiency and availability:

1. ***Lack of an approved and functioning algorithm in Ukraine to establish HIV status to children born to HIV infected mothers, which would envisage virologic testing of blood samples of children (to detect either HIV DNA or HIV RNA, or p24 antigen) to detect HIV infection markers within the first 48 hours after birth***, while the WHO Guidelines recommend such analysis [72]. This test would provide an opportunity to identify children, who were infected in utero, because these very children are in most need in early identification of their HIV status for the timely initiation of treatment, as there are data that in children infected in utero the progression of HIV infection is more aggressive, has more severe clinical manifestations, leads to early development of severe immune deficiency and early mortality [74].

2. ***Discordant, false negative and false positive test results,*** which hinder early and reliable establishment of HIV status of children born to HIV infected mothers (*Tables 7* and *8*). Undoubtedly, these cases require a thorough analysis to identify problems related to the obtaining of false negative and discordant results of test to detect proviral HIV DNA.

It should be noted, that when a laboratory obtains discordant or dubious test result, very often it does not have the capacity to immediately conduct a repeat test because it implies inviting the child again to the Oblast or City AIDS Centre for the repeated blood draw and sending the sample for testing. Usually several months pass between the repeated tests. For example, the laboratory of Ukrainian AIDS Centre had a case

Table 8

Discordant results of laboratory tests to diagnose HIV infection in children born to HIV infected mothers

##	Oblast	Date of birth	First assay to detect proviral DNA		Second assay to detect proviral DNA		Third assay to detect proviral DNA		Result of testing by ELISA, Immune Blot (IB) (confirmation of HIV status), HIV viral load (VL) (if it was detected)	
			Date	Result	Date	Result	Date	Result	Date	Result
1	Dnipro-petrovsk	27.02.2009	*14.04.2009*	*Negative*	*02.06.2009*	*Negative*	–	–	*02.09.2010* *27.10.2010*	***Antibodies to all proteins (IB); VL – 54424 copies/mL****
2		*11.01.2009*	*16.02.2010*	*Negative*	*28.05.2010*	*Negative*	–	–	*05.11.2010* *01.12.2010*	***Antibodies to all proteins (IB); VL – 22768 copies/mL***
3		27.04.2009	02.06.2010	**Positive**	09.07.2010	Negative	21.07.2009	Negative		Taken off the register at the age of 1.5 years
4		16.03.2009	31.03.2009	Negative	27.07.2009	**Positive**	18.08.2009	**Positive**	з 10.10.2009	Receives HAART
5		04.01.2010	19.01.2010	Negative	03.06.2010	**Positive**	08.06.2010	**Positive**	08.06.2010	VL – 54424 copies/mL
6		19.03.2009	19.05.2009	Negative	09.07.2009	**Positive**	21.07.2009	**Positive**	з 05.08.2009	Receives HAART
7		25.05.2009	17.07.2009	**Positive**	28.08.2009	Negative	11.09.2009	Negative	03.12.2010	Negative
8		05.06.2009	23.07.2009	Negative	11.09.2009	**Positive**	24.09.2009	**Positive**	з 09.10.2009	Receives HAART
9		14.02.2010	15.04.2010	**Positive**	01.07.2010	Negative	26.10.2011	**Positive**		Is not eligible to testing by age
10	Kherson	*10.12.2007*	*21.02.2008*	*Negative*	*19.03.2008*	*Negative*	–	–	*17.06.2009*	***Antibodies to all proteins (IB)***
11		*28.02.2009*	*25.06.2009*	*Negative*	*22.10.2009*	*Negative*	–	–	*02.03.2011*	***Antibodies to all proteins (IB)***

Continuation of Table 8

##	Oblast	Date of birth	First assay to detect proviral DNA		Second assay to detect proviral DNA		Third assay to detect proviral DNA		Result of testing by ELISA, Immune Blot (IB) (confirmation of HIV status), HIV viral load (VL) (if it was detected)	
			Date	Result	Date	Result	Date	Result	Date	Result
12	Chernigiv	*02.07.2008*	*13.10.2008*	*Negative*	*08.12.2008*	*Negative*	*21.06.2010*	*Negative*	*19.05.2010* *13.09.2010*	***Antibodies to all proteins (IB); VL – 05 copies/mL***
13	Poltava	*02.09.2008*	*23.04.2009*	*Negative*	–	–	–	–	*30.08.2010* *26.10.2010*	***ELISA-Positive*** ***ELISA-Positive***
14	Zaporizhzhya	24.07.2008	23.10.2008	Negative	22.01.2009	**Positive**	–	–	–	–
15		16.02.2010	25.03.2010	Negative	20.05.2010	**Positive**	–	–	–	–
16	Khmelnytsky	01.06.2009	13.07.2009	Negative	12.10.2009	**Positive**	–	–	06.05.2011	**Antibodies to all proteins (IB)**
17	Kharkiv	17.09.2009	26.09.2009	Negative	22.02.2010	**Positive**	–	–		**Receives HAART**
18	Zakarpattia	23.09.2009	11.06.2010	**Positive**	12.08.2010	Negative	–	–	–	–
19		07.05.2010	11.06.2010	**Positive**	12.08.2010	Negative	13.09.2010	Negative	–	–
20	Donetsk	09.11.2010	23.12.2010	Negative	18.04.2011	**Positive**				Is not eligible to testing by age
21		*09.07.2008*	*04.08.2008*	*Negative*	*20.10.2008*	*Negative*	–	–	*01.02.2011*	***VL – 1158562 copies/mL***
22		02.08.2009	05.10.2009	Negative	02.02.2010	**Positive**	–	–	14.11.2009	VL – 899 copies/mL
23	Cherkasy	*13.10.2007*	*10.04.2008*	*Negative*	*19.06.2008*	*Negative*	–	–	*13.08.2009*	***Antibodies to all proteins (IB)***
24		*05.05.2009*	*13.08.2009*	*Negative*	*08.10.2009*	*Negative*	–	–	*15.11.2010*	***Antibodies to all proteins (IB)***
25		17.06.2009	13.08.2009	Negative	22.10.2009	**Positive**	–	–	22.03.2011	***Antibodies to all proteins (IB)***
26		*24.07.2009*	*12.08.2010*	*Negative*	–	–	–	–	*07.02.2011*	***Antibodies to all proteins (IB)***
27		*08.09.2009*	*22.04.2010*	*Negative*	–	–	–	–	*31.03.2011*	***Antibodies to all proteins (IB)***

Continuation of Table 8

##	Oblast	Date of birth	First assay to detect proviral DNA		Second assay to detect proviral DNA		Third assay to detect proviral DNA		Result of testing by ELISA, Immune Blot (IB) (confirmation of HIV status), HIV viral load (VL) (if it was detected)	
			Date	Result	Date	Result	Date	Result	Date	Result
28	Chernivtsy	11.03.2010	18.05.2010	**Positive**	15.06.2010	Negative	–	–		Is not eligible to testing by age
29	Kyiv, city	01.11.2006	05.02.07	Negative	16.04.2007	**Positive**	07.05.2007	**Positive**	22.04.2008, 24.03.2009, 30.06.2010	**Antibodies to proteins gp160, gp120, p68, p55, p52 (IB)**
30		13.07.2007	13.08.07	**Positive**	27.08.2007	Negative	05.11.2007	Negative		ELISA-Negative
31		11.03.2009	18.06.09	**Positive**	28.08.2009	Negative	10.09.2009	**Positive**	06.11.2009	**VL – more than 10 mln copies/mL. Receives HAART**
32		09.07.2009	26.08.09	**Positive**	10.09.2009	Negative	20.01.2010	Negative		ELISA-Negative
33		16.03.2010	28.04.10	Negative	25.06.2010	**Positive**	06.07.2010	**Positive**		**Receives HAART**
34	Odessa	*10.04.2008*	*10.04.08*	*Negative*	*03.10.2008*	*Negative*	*04.02.2010*	*Positive*	*11.10.2009*	***Antibodies to all proteins (IB)***
35		*25.09.2007*	*08.08.08*	*Negative*	*05.09.2008*	*Negative*	–	–	*14.05.2009*	***Antibodies to all proteins (IB)***
36		*26.04.2009*	*26.11.09*	*Negative*	–	–	–	–	*06.12.2010*	***Antibodies to all proteins (except P18) (IB)***

* HIV viral load value is specified in the number of HIV-1 RNA copies in 1 mL of blood plasma.

when blood samples of an infant were sent for testing twice – on March 20, 2008 and September 16, 2008 – and both times they were rejected due to their poor quality. As a result, the infant was tested after 18 months of age by ELISA assay and turned out to be HIV infected.

3. ***The impossibility of comparing test kit characteristics,*** as Ukraine is using test kits from only one manufacturer, namely, "AmpliSens ® DNA HIV FRT" produced by the Central Research Institute of Epidemiology (Russian Federation), which have been used for early HIV diagnostics in children born to HIV infected mothers since the introduction of this method in Ukraine and until now. Due to this fact it is impossible to assess the sensitivity and specificity of these diagnostic test kits in comparison with the test kits of other manufacturers.

4. ***The lack of any well established system to assure the quality of laboratory tests in the country*** hinders the receipt of the guaranteed quality result of the tests to detect proviral HIV-1 DNA and prevents the early and reliable establishment of the HIV status of children born to HIV infected mothers.

5. ***Irregular supplies of reagents to the laboratories.*** The shelf life of reagents to detect the proviral DNA of the trade mark "AmpliSens" is one year. At the moment of delivery of these products to the laboratories of AIDS Centres their shelf life will be 10 months (in accordance with the technical and medical requirements to the procurement of medical products for the state budget funds, the remaining shelf life of such products should be at least 75%). That is why it is very important to ensure an uninterrupted supply of these reagents to the laboratories so that they could be used to the full and in accordance with the planned needs, i.e., no less than twice a year – in the II and IV quarters..

6. ***Lack of a sufficient amount of equipment***, needed to conduct diagnostic tests prevents further decentralization of testing for early HIV diagnostic in children born to HIV infected mothers.

7. ***Insufficient staffing at interregional laboratories with specialists who have the required skills.*** Testing to detect proviral DNA with the use of test kits manufactured by "AmpliSens" requires a high professional level of the specialists to prepare the sample manually and thus limits the capacity of laboratories to conduct the required number of tests during one day. So, in order to man the laboratories with highly professional specialists there is a need to conduct regular training workshops for laboratory technicians so that they could master the practical and theoretical skills in this area.

8. ***Insufficient funds to pay for the transportation of patients and couriers, who deliver the blood samples to the laboratories.*** On the one hand, this leads to the violation of established terms to test the children, and on the other – it increases the workload of specialists of interregional laboratories due to irregular delivery of blood samples for testing. In general, it results in insufficient coverage for children with this type of testing. That is why there is need to resolve the issue and to find money to pay for the transportation of children and their parents to the regional AIDS Centres for blood drawing and delivery of samples to the interregional laboratories to conduct tests.

9. ***Imperfect system for calculating the indicators of the level of vertical transmission of HIV***, obtained on the basis of the implementation of PCR and ELISA assays – it leads to a significant difference in the indicators calculated on the basis of these techniques. Obviously, this difference is related to an imperfect statistical evaluation of the actual coverage of children with PCR testing. Due to insufficient information about the birth rates and the number of tested children during a year it is difficult to evaluate the actual coverage level: it is not known how many times they were tested. On the other hand, the difference in indicators can be explained by the lack of feedback with the specialists from the regional centres – it is unknown, whether all positive and negative PCR test results were confirmed by ELISA method after children reach the age of 18 months. In general, the drawbacks of the reporting system of the regional AIDS Centres, difficulties to check and control the reported data make it impossible to adequately evaluate the situation related to vertical transmission of HIV in Ukraine. There is a need to improve collaboration between different health care facilities at all levels that are involved in the organization of early HIV diagnostics among children.

Also, an assessment of the expediency of introduction of the use of dried blood spot specimens in the laboratory practice of AIDS service in Ukraine requires special attention. Dried blood spot (DBS) – is a sample of capillary blood dried on a special filter paper card. The advantages of this method in comparison to the venous blood sample include the ease to obtain the sample, lower quality and easier transportation. The use of DBS samples will, probably, help to resolve the existing problems to a certain extent.

The introduction of DBS samples into laboratory practice will, first of all, simplify the blood drawing procedure and will make it possible to perform it at the manipulation rooms of any health care facilities located close to the place of residence of the patients. Besides, thanks to the possibility to send the DBS samples by mail, it will help to resolve the problem with their transportation to the interregional laboratories. The use of DBS will make it possible to conduct testing for proviral DNA within 48 hours after child's birth, which will help to identify children infected in utero as soon as possible. The introduction of the DBS technique into laboratory practice will help to perform repeated tests quickly in case of discordant or dubious test results, and when low quality clinical samples are rejected.

However, the introduction of the DBS technique into laboratory practice for the screening of HIV genetic material requires solutions to a number of issues.

1. Making a decision about the use of new tests, equipment and new testing techniques in Ukraine will be possible, only after a validation pilot trial, which is in line with the current international practice of laboratory diagnostics. The validation of test kits cannot be replaced with the trials that are conducted for the registration of medical products. According to international standards, the validation of the new test kits should be performed before their use in the diagnostic laboratories. International literature confirms that sensitivity and specificity of diagnostic test kits to detect proviral HIV-1 DNA from the DBS specimen is practically the same

as with the use of venous blood samples [29, 61]. However, these studies were conducted with the use of diagnostic test kits that are not used in our country. That is why there is a need to conduct a similar comparison of test kits designed for testing of the whole blood and DBS, which are planned for use in Ukraine.

2. The introduction of the DBS technique into laboratory practice will increase the problem with the deficit of skilled personnel even more. Methods to extract HIV-1 DNA from DBS differ from the methods to extract it from the whole blood specimens, and that is why different samples cannot be processed simultaneously. Due to this fact and taking into account a rather high viral load level, there is a need to study **the possibility to purchase test kits and equipment of other manufacturers that are designed to automatically extract nucleic acids from the clinical specimen.**

3. In spite of the simplified procedures for blood drawing for DBS compared with the process to obtain samples of venous blood, there are still some rules, the violation of which can lead to poor quality of DBS samples and, correspondingly, to inability to use them. **That is why there is a need to conduct workshops and training seminars for the local personnel, who will be involved in the process of blood drawing, storing and transportation of the DBS samples, and in a continuous monitoring of the observance of rules and requirements at the pre-testing stage of the preparation of such clinical samples.**

III. Assessing the Potential Need for the Introduction of DBS Methodology and Costing of Analytical Phase of the Study at the National / Regional Level

1. The Projected Number of Women of Reproductive Age (Absolute Number and Percentage of Women Aged 15–49 Years within the Female Population of Ukraine) and the Number of Live Births by 2030 in Ukraine

Experts from the Institute for Demography and Social Studies after M. Ptukha at the National Academy of Sciences of Ukraine performed multivariate forecasting of the size and age-sex structure of Ukraine's population by 2050 [80], providing rationale for possible development pathways of basic factors that determine the size of the population and its age structure. Therefore, experts projected the number of women of reproductive age (15–49 years) on the basis of forecast that was updated in summer 2010 with relevant accurate data. Authors of the study produced mid-term calculations (by 2030) presenting three possible variations (high, middle and low). Regardless of the scenario, the researchers predict an overall reduction of the number of women of reproductive age; the only difference between variations concerns the extent of possible changes (as consistent with the reduction of the size of general population within the range of 38.4 – 45.7 million). According to the most optimistic scenario, the number of such women may reach 8,941.1 thousand (low variation), 9,571.9 thousand (middle variation), and 9,614.1 thousand (high variation).

Considering the fact that most births occur before 35 years of age (according to official statistics, 91.2% of babies in 2010 were born to mothers younger than 35 years, and only 8.8% were born to women of older age), we also calculated possible changes in the size of female population aged 15–34 years. By 2030 the number of "potential mothers" is expected to range between 4,628.12 and 4,275.68 thousand persons. It is known that the risk of HIV exposure is particularly high for women of younger age categories of reproductive period.

In addition, the researchers calculated possible changes in the proportion of this contingent within the overall female population. It is expected that by 2030 from 39.96% to 43.63% of all women in Ukraine will be in their reproductive years (*Fig. 5*).

We also analysed potential changes in the number of births (*Fig. 6*), as all variations of the forecast show gradual reduction of the number of newborn children.

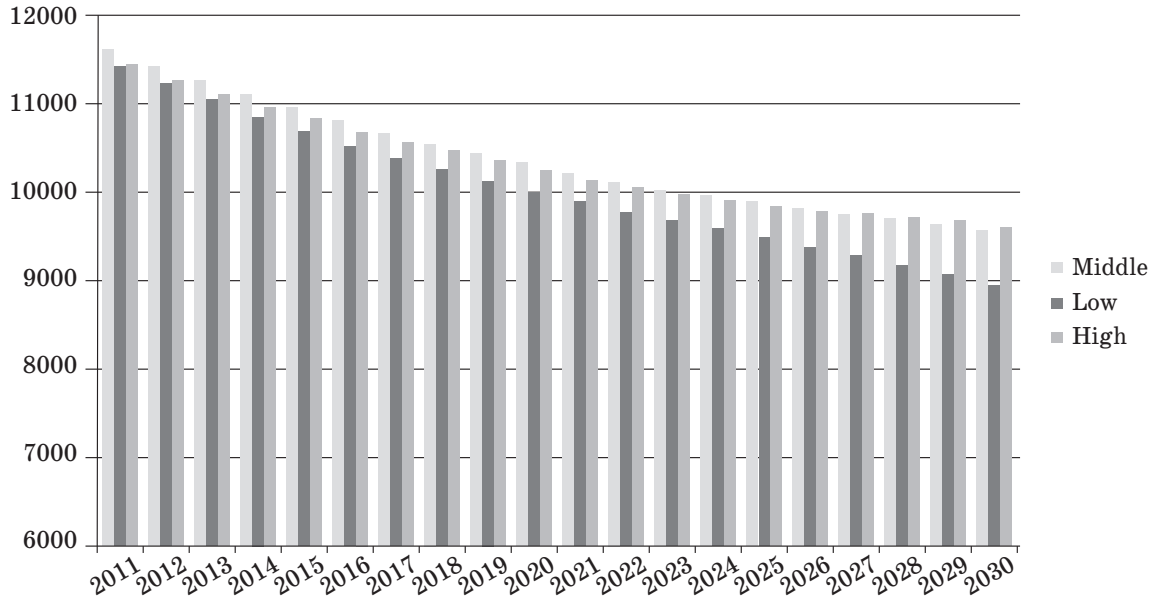

Fig. 4. **Projected number of women of reproductive age (15–49 years) in Ukraine by 2030, according to high, middle and low forecast variations,** *thousand persons*

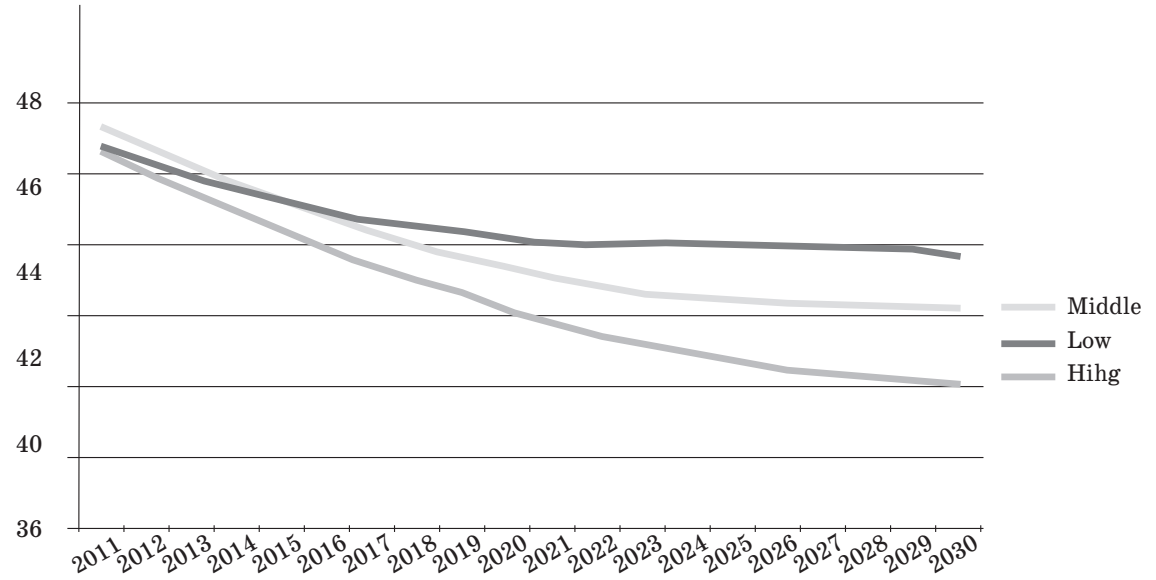

Fig. 5. **Projected proportion of women aged 15–49 years in the overall female population of Ukraine by 2030, according to high, middle and low forecast variations,** %

A further spreading of the HIV epidemic may also become a determinant in the expected decline in birth rates, as many specialists [82] predict possible reduction of fertility among HIV positive women up to 30%.

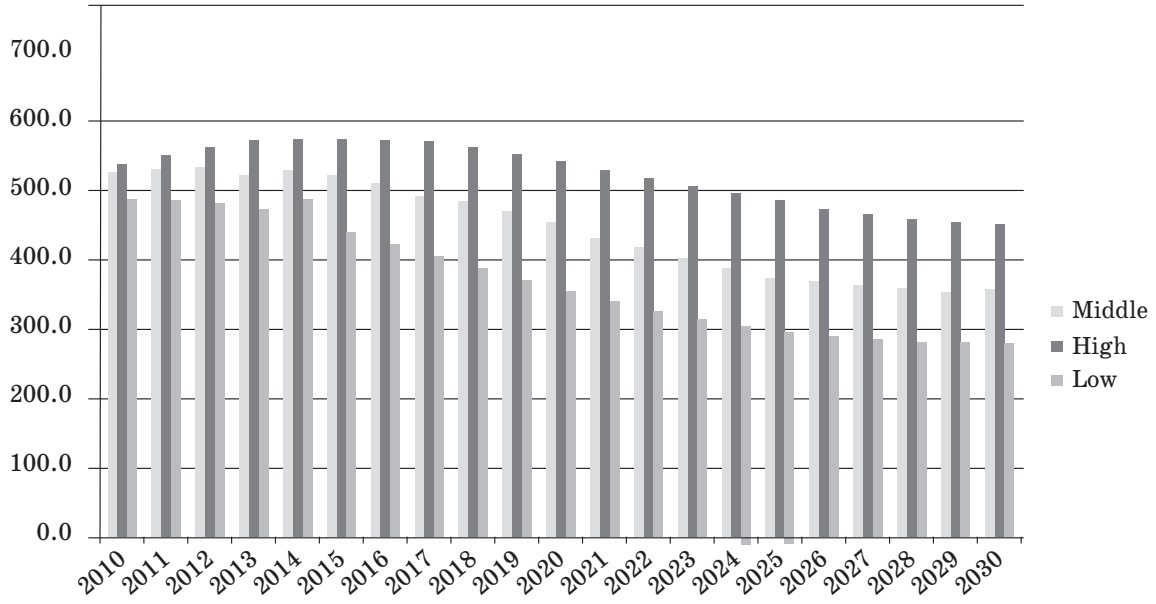

Fig. 6. **Estimated number of live births in Ukraine by 2030, according to high, middle and low forecast variations,** *thousand persons*

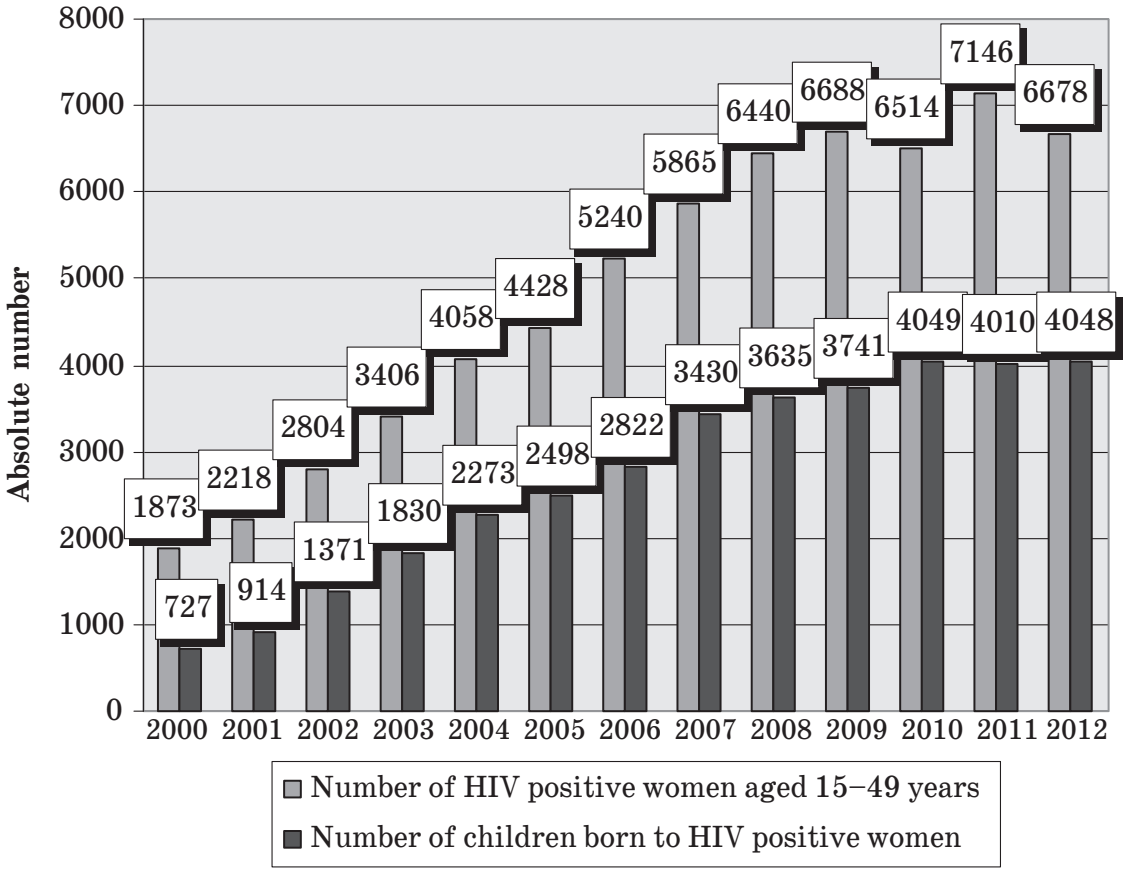

Fig. 7. **Dynamics of the number of HIV positive women of reproductive age and children born to them in Ukraine**

2. An Assessment of the Projected Numbers of HIV Positive Women of Reproductive Age and Children Born to Them

Statistics shows that increasing rates of sexual transmission for HIV and a growing number of fertile HIV positive women contributes to gradual increase of the number of children born to HIV positive women (*Fig. 7*).

According to the research results from the "Projected Mid- and Long-Term Economic Consequences of HIV/AIDS Epidemic" study, carried out by the Institute for Economics and Forecasting (IEF, 2008) [86], the overall number of HIV positive individuals in 2008 was estimated within the range of 477 – 485 thousand, whereas the estimated infection rate in the age group of 15–49 years was 1.85% (pessimistic scenario) and 1.81% (optimistic scenario). Forecast data until 2014 are presented in *Table 9*.

Table 9

Projected number of HIV positive individuals and HIV prevalence among the adult population

	2010	2011	2012	2013	2014
Pessimistic scenario					
Total	544 599	591 195	639 246	674 870	709 039
Men	290 496	311 325	333 705	347 879	362 476
Women	254 103	279 870	305 541	326 991	346 563
HIV prevalence (15–49 years), %	2.08	2.28	2.49	2.66	2.82
Optimistic scenario					
Total	507 105	531 456	557 536	570 827	582 416
Men	270 834	280 446	291 908	295 314	298 936
Women	236 271	251 009	265 628	275 513	283 479
HIV prevalence (15–49 years), %	1.92	2.03	2.14	2.21	2.28

By using both optimistic and pessimistic assumptions, and relying upon the middle variation of multivariate forecast of the overall size and sex-age structure of the Ukrainian population by 2050, it is possible to estimate the number of HIV positive women of reproductive age in 2014 (one should expect the reduction of the number of women of this age category in the upcoming years as compared to 2010) as ranging from 6 – 7 thousand (which is extremely unlikely even in the most optimistic assumptions concerning the course of the epidemic) to 283–346 thousand persons.

Accordingly, the proportion of children born to these women will also increase, because with further spread of the epidemic beyond the most-at-risk populations and with growing numbers of women living with HIV, the share of pregnant women who may choose to give birth to children and to raise them in families will increase. The generalization of a HIV epidemic changes psychological "profile" of an HIV positive pregnant woman and the share of women from most-at-risk populations who would

often choose not to keep a baby (including due to living conditions) is declining, whereas the proportion of more "successful" positive women who are likely to keep their pregnancies and raise children in families is increasing.

Better awareness amongst the population regarding ways of preventing mother-to-child transmission of HIV and giving birth to a healthy baby also contributes to the situation. Findings of the nationally representative Ukraine Demographic and Health Survey (UDHS 2007) covering individuals of 15–49 years from all regions of the country show that 60% of female and 39% of male respondents knew that HIV can be transmitted by breastfeeding, while 33% of women and 31% of men were aware of the fact that the risk of MTCT could be reduced by the mother taking special drugs during pregnancy. In general, women are more likely than men to know ways of preventing mother-to-child transmission of HIV, which confirms the effectiveness of work of health and social workers with pregnant women [85].

On the assumption of consistency, the current correlation between the total number of new-borns in Ukraine and babies born to HIV positive mothers (about 0.8%), "conservation" of current epidemiological situation (which is absolutely unreal), and the realization of present-day expectations regarding basic demographic trends, one can expect that until 2020 HIV positive mothers will be giving birth to between 2.5 and 4 thousand babies annually, even taking into account low birth rate forecasts. If prognoses of IEF specialists on further spread of HIV among women of reproductive age come true, the number of such children can be several times higher.

According to experts [4], the successful implementation of comprehensive systems of evidence-based prevention measures for all HIV positive pregnant women (including specific chemoprophylaxis with ARVs, selection of optimal birth management, and the refusal of breastfeeding) will make it possible to reduce the risk of vertical transmission to a minimum, yet HIV still can be transmitted from a mother to a child in less than 2% of cases. Sharing a belief that "by 2015 children everywhere can be born free of HIV", expressed by Michel Sidibe, Executive Director of UNAIDS, at 2011 United Nations General Assembly High Level Meeting on AIDS in New York, we still understand the persistent need in early and reliable identification of HIV status of babies born to HIV positive mothers.

3. Cost Estimates for Reagents and Expendables to Perform the Analytical Phase of DBS Testing

In order to estimate the supplies required to perform the analytical phase of DBS testing according to procedure, established by the MOH Order No. 740 as of November 23, 2007 (typically two tests, and in case of discordant results – three tests before 18 months of age), we suggest the following working formula:

$$F = S \times (2 + k_1) \times (1 + k_2),$$

where:

F is the cost of analytical phase of testing of one child born to HIV positive mother;
S is the cost of reagents and expendable materials per one DBS test;
k_1 is the percentage of discordant results (specified depending on MTCT rates);
k_2 is the percentage of discarded samples inconsistent with quality assurance criteria (2% on average).

Observations, reflected in WHO guidelines on early HIV diagnosis in new-borns, show that the proportion of discordant results between the first and the second test depend on the transmission rates in the regions [72].

Overall in Ukraine about 2% of tests have discordant results, in other words k1 is 0.02. The number of discordant results also depends on early diagnosis scenario. If they opt to perform initial testing at the maternity settings, then the number of discordant results may be higher compared to other scenarios, as it is well known that 20 to 40% of HIV positive babies (particularly those infected during delivery, and with transmission rates of 5% – in one or two babies per 100) will show true negative results of the first test within 48 hours after birth that will become true positive by the end of the first month.

As a rule of thumb, almost 2% of samples on average are inconsistent with quality criteria. Therefore, k_2 does not exceed 0.02.

Accordingly, the cost of the analytical phase of the testing of all children born to HIV positive mothers during a given year and before attainment of 18 months of age would be $S \times (2 + k_1) \times (1 + k_2)$. In average measure suggested formula will be as follows:

$$S \times (2 + k_1) \times 1{,}02.$$

If we use any other algorithm for introducing DBS, this formula can be adequately adjusted by changing the multiplicity of tests.

A comparison table for calculating the cost of reagents and expendable materials for tests at analytical phase using both whole blood and DBS specimens is presented below (identical expenditures for both methodologies are presented in italic: UAH 98.42 when using comparatively expensive systems, and UAH 33.3 when using systems in Option 2).

The working group suggested disregarding such expenditures as depreciation of equipment and the time used by a health worker to process one test, because these

costs were not included in the procurement planning for other testing methodologies. The suggestion was to include a "material section" only, that is, materials for the analytical phase of DBS testing (filter paper, dehumidifying elements, packs and the like) and a tentative price for test kits. Calculations were made for testing of 80 children in 1,000, including controls.

Table 10

Comparative table of the cost of reagents and expendables to perform analytical phase of tests

Whole blood			**DBS method**		
Type of reagents / and expendables	**Necessary quantity**	**Total cost,** *UAH**	**Type of reagents / and expendables**	**Necessary quantity**	**Total cost,** *UAH*
Test kit	100	840.00 / or 6,050.00	Test kit	100	840.00 / or 6,050.00
Eppendorf test tubes, 1.5 ml	100	197.00	Eppendorf test tubes, 1.5 ml	100	197,00
PCR tubes, 0.2 ml	100	55.00	PCR tubes, 0.2 ml	100	55.00
Universal dispensing tips for automatic dispensers:			Universal dispensing tips for automatic dispensers:		
• 1000 μl, with filter	8	864.00	• 1000 μl, with filter	8	864.00
• 200 μl, with filter	3	324.00	• 200 μl, with filter	3	324.00
• 200 μl, without filter	8	384.00	• 200 μl, without filter	8	384.00
Vacutainers with K3EDTA, holders and butterfly needles	80	636.00	Eppendorf test tubes **	100	197.00
			Universal dispensing tips for automatic dispensers, 1,000 μl, with filter	8	864.00
			Cards (filter paper)	0.8	2,100.80
			Dehumidifying elements	0.8	32.00
			Bags with zip fastener	0.08	5.36
			Parchment paper for packing cards with samples	0.88	4,224.00
			Moisture indicators	1.6	2,352.00
Total		**3,300**	**Total**		**12,439.20**
Cost per test		**41.30** / *or 106.00*	**Cost per test**		**155.50** / *or 220.60*

* Prices in Ukrainian hryvna in 2011.

** While using DBS sample the first step of the analysis is extraction of blood sample from paper, which requires additional tube and tip.

Testing also requires a hole punch for extracting samples and a shaker, but these were not included in the table as non-disposable laboratory equipment.

Note. The cost of bags with zip fasteners was calculated on the basis of the cheapest offer, but the price is subject to change following specification of the necessary quantity of these items. In this option we calculated one bag per sample, but according to the protocol one can store 5 to 10 Whatman 903® cards in a single hermetically sealed plastic bag, therefore the demand for this product will be lower. The remaining bags will be used anyway when there is a need to replace a bag in case of excessive moisture (moisture indicator becomes pink) or damage of the bag.

Initial calculations were made with applications available online.

Table 11

Cost estimates for reagents and expendables to perform analytical phase of DBS tests (Option 1)

Type of reagent / expendable material	**Unit**	**Unit cost,** *UAH*	**Need (units)**	**Total cost,** *UAH*
Test kit	Set, 96 tests	6,050.00	1	6,050.00
Eppendorf test tubes, 1.5 ml	Piece	1.97	100	197.00
PCR tubes, 0.2 ml	Piece	0.55	100	55.00
Universal dispensing tips for automatic dispensers:				
• 1000 μl, with filter	Rack, 100	108.00	8	864.00
• 200 μl, with filter	Rack, 96	108.00	3	324.00
• 200 μl, without filter	Rack, 96	48.00	8	384.00
Eppendorf test tubes	Items	1.97	100	197.00
Universal dispensing tips for automatic dispensers, 1000 μl, with filter	Rack, 96	108.00	8	864.00
Whatman 903® cards	100 pack	84,160.00	0.8	67,328.00
Dehumidifying elements	100 pack	10,560.00	0.8	8,448.00
Plastic bags with zip fastener	100 pack	14,400.00	0.08	1,152.00
Foil paper for packaging	100 pack	23,600.00	0.88	20,768.00
Moisture indicators	1000 pack	1,470.00	1.6	2,352.00
Total				108,983.00
Cost per test				**1,362.29**

However, such excessively high costs forced us to seek other sources; moreover, many research publications cited the particularly low cost of DBS testing (according to various specialists [26, 43], expenditures on the collection of DBS samples are less than one US dollar per test).

Therefore, for the second option we used different price offers for cards, dehumidifying elements, moisture indicators and bags. Substituting foil with cheaper parchment paper reduces the cost by several times. As a result, the cost of one test has dropped to only UAH 155.5 (see *Table 12*).

Table 12

Cost estimates for reagents and expendables to perform analytical phase of DBS tests (Option 2)

Type of reagent / expendable material	Unit	Unit cost, *UAH*	Need (units)	Total cost, *UAH*
Test kit	Set, 96 test	8.40	1	840.00
Eppendorf test tubes, 1.5 ml	Piece	1.97	100	197.00
PCR tubes, 0.2 ml	Piece	0.55	100	55.00
Universal dispensing tips for automatic dispensers:				
• 1000 μl, with filter	Rack, 100	108.00	8	864.00
• 200 μl, with filter	Rack, 96	108.00	3	324.00
• 200 μl, without filter	Rack, 96	48.00	8	384.00
Eppendorf test tubes	Items	1.97	100	197.00
Universal dispensing tips for automatic dispensers, 1000 μl, with filter	Rack, 96	108.00	8	864.00
Filter cards	100 pack	2,626.00	0.8	2,100.80
Dehumidifying elements	100 pack	40.00	0.8	32.00
Plastic bags with zip fastener	1,000 pack	67.00	0.08	5.36
Parchment paper for packaging	100 pack	4,800.00	0.88	4,224.00
Moisture indicators	1,000 pack	1,470.00	1.6	2,352.00
Total				12,439.20
Cost per test				**155.50**

If we calculate the cost of materials for the pre-analytical phase of DBS testing in line with the needs of the pilot project, on the one hand we can exclude the cost of test systems that are provided by the Russian company "AmpliSens" free of charge; on the other hand we incur additional expenditures on vacutainers with K3EDTA, holders and butterfly needles, as it is necessary to collect venous blood into closed blood sampling system (which usually consists of vacutainer tubes with K3EDTA, holders and butterfly needles).

Therefore, by using this suggested calculation algorithm and assessing the need on the basis of the number of babies, born to HIV positive mothers in the country or in specific region, MTCT rates and selected early diagnosis scenario, as well as specific cost of reagents and expendables, it is possible to calculate overall expenditures on DBS testing at the national or regional level.

Table 13

Cost estimates for reagents and expendables to perform analytical phase of DBS tests (Option 3)

Type of reagent / expendable material	**Unit**	**Unit cost,** *UAH*	**Need (units)**	**Total cost,** *UAH*
Vacutainers with K3EDTA, holders and butterfly needles	Set, 96 test	7.95	80	636.00
Eppendorf test tubes, 1.5 ml	Piece	1.97	100	197.00
PCR tubes, 0.2 ml	Piece	0.55	100	55.00
Universal dispensing tips for automatic dispensers:				
• 1000 µl, with filter	Rack, 100	108.00	8	864.00
• 200 µl, with filter	Rack, 96	108.00	3	324.00
• 200 µl, without filter	Rack, 96	48.00	8	384.00
Eppendorf test tubes	Items	1.97	100	197.00
Universal dispensing tips for automatic dispensers, 1000 µl, with filter	Rack, 96	108.00	8	864.00
Filter cards	100 pack	2,626.00	0.8	2,100.80
Dehumidifying elements	100 pack	40.00	0.8	32.00
Plastic bags with zip fastener	1,000 pack	67.00	0.08	5.36
Parchment paper for packaging	100 pack	4,800.00	0.88	2,352.00
Moisture indicators	1,000 pack	1,470.00	1.6	2,352.00
Total				12,235.16
Cost per test				**152.93**

IV. Pilot Research Protocol "Introducing Dried Blood Spot (DBS) Technique to Improve Early Diagnosis of HIV in Newborns"

M. H. Liulchuk

This protocol of the research project "Evaluation of the performance of Roche COBAS Ampliprep / COBAS Taqman HIV-1 Qual, Abbott Real-Time HIV-1 Qualitative, AmpliSens® DNA-HIV-FRT, and HIV-1 Ultrasensitive p24 ELISA for Early Infant Diagnosis using Dried Blood Spots (BDS) in Ukraine" was developed within the framework of cooperation of the Ukrainian AIDS Centre of the Ministry of Health of Ukraine, United Nations Children's Fund (UNICEF) in Ukraine and Centers for Disease Control and Prevention (CDC).

General Objective

To evaluate the performance of four platforms: CAP-CTM HIV-1 Qual (Roche), Abbott Real-Time HIV-1 Qualitative, AmpliSens ® DNA-HIV-FRT PCR and HIV-1 ultrasensitive p24 ELISA (PerkinElmer) using DBS.

Specific Objectives

1. To compare the sensitivity and specificity of four platforms, CAP-CTM HIV-1 Qual (Roche), Abbott Real-Time HIV-1Qualitative, AmpliSens® DNA-HIV-FRT PCR (Russia), and HIV-1 ultrasensitive p24 ELISA (PerkinElmer) using DBS with the sensitivity and specificity of AmpliSens® DNA-HIV-FRT PCR using EDTA whole blood.

2. To compare the analytical sensitivity or limit of detection (LOD) of four platforms using DBS specimen with the limit of detection of AmpliSens® DNA-HIV-FRT PCR using whole blood as specimen.

3. To evaluate the reproducibility of each platform using DBS as a specimen source.

Design and Methodology

The protocol is designed to evaluate the performance of four platforms, namely CAP-CTM HIV-1 Qual (Roche), Abbott Real-Time HIV-1, AmpliSens® DNA-HIV-FRT PCR (Russia), and HIV-1 p24 ELISA (PerkinElmer), using DBS as specimen for EID and comparing their performances with the AmpliSens® DNA-HIV-FRT PCR using whole blood.

Primary HIV diagnosis of infants is being conducted at the six regional AIDS Centres and the DBS preparation and storage are ensured by the Ukrainian AIDS Centre of the Ministry of Health of Ukraine. The Ukrainian AIDS Centre laboratory has accreditation from the Central Organization of Metrology and Standardization of Ukraine at the National Specialized Children's Hospital "OKHMATDYT", a national body authorized for accreditation of laboratories in Ukraine.

This protocol is a non-research determination protocol. The blood specimens that will be used to produce DBS cards for this study will be derived from leftover samples from infants (under 18 months of age) whose whole blood samples (current Ukraine's standard EID sample type) are taken for HIV diagnosis at 6 different oblast sites (AIDS Centres) in Ukraine.

The use of DBS on Roche CAP-CTM, Abbott M2000, AmpliSens® DNA-HIV-FRT PCR, and p24 ultrasens ELISA (PerkinElmer) assay will be evaluated using broad guidelines provided under the Clinical Laboratory Improvement Amendments (CLIA) guidelines for moderate or high complexity tests. Such evaluations will include:

a. Assay sensitivity and specificity of 4 platforms using DBS as specimen;

b. Analytical sensitivity or limit of detection of 4 platforms using DBS;

c. Reproducibility of 4 platforms or assays using DBS.

Study population

Currently, whole blood is used for EID in Ukraine. The blood specimens for this protocol used to prepare the DBS will be derived from leftover blood drawn intravenously from the infants (under 18 months of age), who come to AIDS Centres for HIV diagnosis. A total of 6 regional AIDS Centres (Crimean republican, Dnipropetrovsk, Donetsk, Mykolayiv and Odessa oblast, and Kyiv city) will participate.

Specimen Inclusion Criteria

1. Leftover whole blood derived from infants who have two independent and matching EID test results from Oblast AIDS Centres will be included: infants must have either 2 HIV positive or 2 negative HIV test results by current EID method (AmpliSens® DNA-HIV-FRT PCR using whole blood). According to Ukraine EID algorithm, confirmatory test is completed approximately 3 months after primary testing for HIV negative infants, but within 1–2 weeks for HIV positive infants.

2. A sufficient quantity of leftover blood of about 800 ?L is necessary to generate 10 DBS spots.

3. Only whole blood specimens that are collected using EDTA tubes will be accepted for this study.

4. Under the age of 18 months.

5. Permanently live in one of 6 oblast sites.

Specimen Exclusion Criteria

1. Rejecting blood specimens that were collected using other tubes rather than EDTA tubes.

2. Rejecting blood specimens that present any sign of contamination, e.g. mould or bacteria growth.

Sample Size and Duration of the Study. In order to achieve statistical significance for the comparison of the AmpliSens® DNA HIV-FRT PCR using whole blood with the 4 assays, a minimum of 100 HIV positive and 100 HIV negative DBS samples will need to be tested on each platform. The HIV positive and negative samples will be collected based on the HIV diagnosis determined by the AmpliSens® DNA-HIV-FRT PCR using whole blood.

Each participating AIDS centre (research site) should provide at least 40 blood samples, including:

- **20 samples** from children born to HIV positive mothers with identified **presence** of HIV proviral DNA;
- **20 samples** of children born to HIV positive mothers with identified **absence** of HIV proviral DNA.

Overall it is expected to obtain at least 240 samples of blood in six selected research sites.

Sentinel Sites: The study is carried out in Crimean, Dnipropetrovsk, Donetsk, Mykolayiv, Kyiv and Odessa regions. Specific sites, where actual collection of blood samples of children, born to HIV positive mothers, shall occur include polyclinic departments of Crimean Republican AIDS Centre, Dnipropetrovsk, Donetsk, Mykolayiv and Odessa oblast AIDS centres, and Kyiv City AIDS Centre, where children under study stay under dispensary observation and receive medical assistance.

Sample Collection. It is envisaged that local research coordinator at each site will organize sampling of peripheral (venous) blood in children who comply with the sampling criteria. While in sterile environment, each participating child will provide 3 ml of blood from peripheral vein to be collected in closed blood sampling systems (such systems consist of vacutainer with K3EDTA, holders and butterfly needles). If at least 800 μl of whole blood remains, then each participating regions will transport 20 HIV positive and 20 HIV negative EDTA tubes to the Ukrainian Centre for Control of Socially Dangerous Diseases of the Ministry of Health of Ukraine. During the process of blood sampling it is strictly prohibited to open the caps – blood should pour in the vacutainer due to system's internal vacuum.

Test tubes with collected blood should be upended gently several times in order to mix the blood with EDTA; then – within 24 hours and in conditions of cold chain – tubes are transferred to the Reference Laboratory of the Ukrainian Centre for Control of Socially Dangerous Diseases of the Ministry of Health of Ukraine. Once the whole

blood specimens arrive at the Ukrainian Centre for Control of Socially Dangerous Diseases, a minimum 10 DBS spots (70 μl blood/spot) will be spotted on Whatman 903 filter paper immediately and appropriately labelled with unique identifier. DBS samples should be allowed to dry for at least 24 hours in the indoor temperature (about 25°C).

Samples of whole blood without accompanying papers (or with incorrectly filled documents) shall be excluded from the research. Primary responsibility for the quality of samples and accompanying documentation shall be borne by the local research coordinator, appointed by the head of participating AIDS Centre.

Storage and Transportation of Samples. All whole blood samples shall be collected and delivered in cooling boxes with cooling elements to the Reference Laboratory of the Ukrainian Centre for Control of Socially Dangerous Diseases of the Ministry of Health of Ukraine within 24 hours.

Dried filters need to be interleaved with parchment paper (if filters do not have wrapping of their own) and stacked together in hermetically sealed plastic bags with 5–10 filter papers in each. For DBS packaging plastic bags with zip fasteners are used. Material of the bag should be airproof (food packages and bags are not suitable). Then they put several dehumidifying elements inside to get rid of excessive moisture, as well as moisture indicator card in every bag.

Properly prepared DBS samples can be stored in indoor temperature (from +15°C to +30°C) during two weeks (in case of the absence of direct light). For more lengthy storage (up to 6 months) it is necessary to put DBS samples in the freezer with the temperature of −20°C.

During the storage the moisture indicator needs to be monitored closely: every day during the first week, and weekly during subsequent storage. If an indicator changes colour to pink, filters should be repacked in another plastic bag with fresh dehumidifying elements and new moisture indicator card (excessive moisture impairs the quality of testing results). If the storage bag gets damaged, it should be immediately replaced. Storage control data for DBS samples should be maintained in the special log.

The testing of DBS samples for HIV proviral DNA to evaluate the performance of four platforms, namely Roche COBAS Ampliprep / COBAS Taqman HIV-1 Qual, Abbott Real-time HIV-1 Qualitative, AmpliSens ® DNA-HIV-FRT and HIV-1 Ultrasensitive p24 ELISA for early HIV diagnosis in new-borns with DBS methodology in Ukraine shall be carried out in the laboratories of the US Centers for Disease Control and Prevention (CDC). DBS samples shall be delivered in two stages – in August 2012 and in May 2013 after collection of all samples suitable for testing.

Data Confidentiality

Only a minimum set of data for each sample, obtained from an HIV positive child, should be included in the sample registration card. Such card should not be included in the patient's clinical or health records. Results of DBS testing SHALL NOT be communicated to the child's parents.

An electronic database containing testing results should be password-protected, with access granted exclusively the local project coordinator. Results of the study shall be used in generalized form for reporting and informing relevant heads of healthcare institutions and facilities and managers of HIV/AIDS programmes about situation with early diagnosis in the country, for preparation of reports and for policy development. The data cannot be used for individual patient monitoring.

Sample-related Information

The National Reference Laboratory of the Ukrainian Centre for Control of Socially Dangerous Diseases of the Ministry of Health of Ukraine has developed its own database of samples, received from pilot sites (regional AIDS centres), using the data from the sample registration cards. The following data is included in standard registration card:

- Sample number / code;
- Child's date of birth;
- Child's gender;
- Dates and results of previous two tests to identify HIV proviral DNA in the whole blood samples;
- Institution / facility where the sample was collected;
- Child's place of residence;
- Date and time of blood sampling;
- Date of refrigeration and storage temperature;
- Date of transportation.

Local coordinators from AIDS centres involved in the project implementation immediately fill registration cards. Local coordinators shall be fully responsible for the completeness, reliability and confidentiality of information, presented in the cards.

Setting up the National Database

It is expected that the national lab, responsible for the storage of DBS samples, will develop and maintain relevant database to include child-related data and results of his/her previous laboratory examinations. The database should also include information about the date of the data entry and persons responsible.

Observation over DBS Sampling Process, Analysis and Dissemination of Research Results

Laboratory experts shall be responsible for the quality of research at every stage. This specialist shall cooperate with local coordinators to prevent any systemic errors during the blood collection from patients involved in the study. Quality assurance of blood samples should be discussed with relevant staff to prevent errors at the stage of selection of children and DBS preparation.

Detection of HIV proviral DNA during testing of BDS samples, collected among HIV positive babies under 18 months of age, will make it possible to validate specifically developed BDS platforms; to evaluate the effectiveness of sampling of biological material (in the form of dried blood spots); and, in case of positive outcomes – to decide on introduction of this technology in Ukraine with the goal of improving current system of early HIV diagnosis in children, born to HIV positive mothers.

At the end of the study in 2013 the final report will provide generalized data to inform decision-makers, heads of relevant healthcare institutions and facilities, as well as managers of HIV/AIDS programmes on the situation with early HIV diagnosis in new-borns, as well as to foster development of future strategies.

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Annex

The State Facility "Ukrainian Centre for Control of Socially Dangerous Diseases at the Ministry of Health of Ukraine"

The Project "Improvement of Early HIV Diagnostics in Children Born to HIV Positive Mothers through Introduction of Dried Blood Spot (DBS) Technique"

Sample Registration Cards No. _____

1.1. Child's gender: ☐ male ☐ female

1.2. Child's date of birth: day _____, month _____, year _____

1.3. Place of child's permanent residence:

_____; _____;
city village

1.4. Institution/facility where the sample was collected _____

_____

1.5 Results of previous two tests to identify HIV proviral DNA in the **whole blood** samples

The date of the 1st testing for the presence of HIV-1 proviral DNA _____

Result: ☐ positive ☐ negative

The date of the 2nd testing for the presence of HIV-1 proviral DNA _____

Result: ☐ positive ☐ negative

1.6. **Sample-related Information**

Date _____ and time _____ of blood sampling

Temperature of storage:

☐ at +20°C; storage life from _____ to _____ 20___

☐ at +4°C…+8°C; storage life from _____ to _____ 20___

Date of freezing (if appropriate): ☐☐/☐☐/☐☐
day/month/year

Temperature of storage: ☐ −20°C ☐ −70°C

Date of transportation to the Reference Laboratory : ☐☐/☐☐/☐☐
day/month/year

Date of receiving of the sample by the Reference Laboratory : ☐☐/☐☐/☐☐
day/month/year

Signature of responsible person / local coordinator _____

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