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ABSTRACT

Early diagnosis of HIV in infants is critical because it can remarkably impact an infant's survival. DNA PCR is the standard test for diagnosis of HIV-1 in infants and young children less than 18 months of age. For settings that lack the adequate infrastructure for processing whole blood and cold-chain transportation, the collection of dried blood spots (DBS) has facilitated the detection of HIV-1 in infants as early as 4-6 weeks after birth. Molecular testing using DBS provides an accurate method for the identification of HIV-1 but quality testing depends greatly on adequate quality assurance. A voluntary, cost-free external quality assurance program established by the U.S. Centers for Disease Control and Prevention, Global AIDS Program was implemented to monitor the performance of laboratories conducting HIV EID from DBS in an effort to provide the critically needed external quality assurance measures in resource-constrained settings. Known HIV-positive and negative DBS specimens to be used as internal controls and ten blinded DBS specimens are shipped internationally tri-annually with a 30 day testing result turnaround. Peer comparison is provided after each testing time point. Advances by resource-constrained countries to conduct EID have resulted in more children being tested, which resulted in enrollment and participation expanding significantly to include greater than 104 laboratories from 36 countries. Mean test scores have improved with each testing but false negative results are twice as likely as false positive discordant outcomes.

EVALUATION OF PROFICIENCY TESTING PROGRAM FOR LABORATORIES
CONDUCTING HIV-1 DNA DETECTION FOR EARLY INFANT DIAGNOSIS FROM
DRIED BLOOD SPOT SPECIMENS IN RESOURCE-LIMITED SETTINGS

By

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2002

A Thesis Submitted to the Graduate Faculty of Georgia State University in Partial Fulfillment of
the Requirements for the Degree

Master of Public Health

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APPROVAL

for

EVALUATION OF PROFICIENCY TESTING PROGRAM FOR LABORATORIES
CONDUCTING HIV-1 DNA DETECTION FOR EARLY INFANT DIAGNOSIS FROM
DRIED BLOOD SPOT SPECIMENS IN RESOURCE-LIMITED SETTINGS

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DEDICATION

The following document is dedicated to my family who provided the network of support which allowed me to see this task to completion. Thank you for your love and patience.

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Albert D. Garcia
2013

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I would like to personally acknowledge Dr. Dennis Ellenberger and Dr. Ike Okosun for their continuous guidance through this project. I would also like to thank Dr. Ellenberger, Dr. Shambavi Subbarao, Dr. Linda Parsons and Dr. Chin-Yih Ou for their contributions to the development of the Early Infant Diagnostics Proficiency Testing Program and acknowledge them as co-authors of the manuscript that will be submitted as a result of this work.

AUTHOR'S STATEMENT

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CHAPTER I - INTRODUCTION

Since 2006, the Division of Global HIV/AIDS of the Centers for Disease Control and Prevention (CDC) has provided a cost-free proficiency testing program for complex molecular laboratory tests used in early infant diagnosis (EID) of human immunodeficiency virus type 1 (HIV-1) in resource-limited settings (RLS). During this time period the number of participants has increased consistently over time. This thesis will show a positive trend in participant's mean score over time. The rationale for this project is to show that proficiency testing programs will improve laboratory performance and improve the quality of testing. This study hopes to show the utility of a proficiency testing program for EID of HIV-1 by demonstrating a positive correlation between participation and performance by laboratories participating in the EID of HIV-1 EQA program.

BACKGROUND

Worldwide, children account for nearly 1 in 5 of all HIV-related deaths and 1 in 6 of new HIV infections each year. More than 90% of the annual 400,000 pediatric HIV infections globally are acquired vertically through mother-to-child transmission (MTCT) (UNAIDS 2010), and most of these vertical transmissions occur in sub-Saharan Africa. MTCT occurs during pregnancy, intrapartum period (labor and delivery) and breastfeeding. Without active intervention efforts, approximately 15-30% of babies born to the HIV-infected mothers will become infected with HIV during pregnancy and delivery. An

additional 5-20% will become infected through breastfeeding (DeCock 2000). Prevention of mother-to-child transmission (PMTCT) programs provide interventions such as antiretroviral prophylaxis and treatment for mothers, prophylaxis for infants, and avoidance of breastfeeding to reduce infant infection of HIV by 1-20%. (Nolan 2002; Lallemand 2004). Early infant diagnosis (EID) of HIV in infants can serve as indicators to monitor the efficacy of PMTCT programs in .

EID is critical because it can remarkably impact an infant's survival. If the child receives proper access to medical treatment then the early diagnosis and treatment of the HIV infection improves outcomes for infected infants and reduces early mortality and morbidity (Berk 2005). The South African CHER study reported the survival benefit of early treatment for infants is an overall 75% reduction in infant mortality in those infants that were treated immediately after diagnosis before 12 weeks of age (Violari 2008). Untreated HIV-infected infants have extremely high mortality with nearly 20% dying before the age of 6 months, 35-40% by the age of 1 year, and 50-60% by 2 years and reaching 80% by 5 years of age (Obimbo 2004; Newell Lancet 2004; Newell AIDS 2004; Brambilla 2003).

Routine HIV diagnostic testing such as highly accurate rapid tests and enzyme immunoassays that are designed for older children, adolescents and adults do not meet the needs of infants and young children (<18 months). Routine serologic testing of infants is generally only informative before the age of 18 months if the test result is negative. Virologic assays, including HIV-1 DNA or RNA assays, represent the gold standard for diagnostic testing of infants and children younger than 18 months. With such testing, the diagnosis of HIV-1 infection (as well as the presumptive exclusion of HIV-1

infection) can be established within the first several weeks of life among non-breastfed infants. WHO recommends that diagnostic testing with HIV-1 proviral DNA or viral RNA assays be performed within the first 14 days of life, at 1 to 2 months of age, and at 3 to 6 months of age (WHO). If any of these virologic test results are positively reactive then repeat testing is recommended to confirm the HIV diagnosis. In developed countries, HIV DNA PCR is the preferred test for confirming the diagnosis of HIV infection in children less than 18 months of age and is frequently repeated. However, in resource-limited settings a single HIV DNA PCR is completed frequently without repeating thus the quality of the testing is critical.

CDC researchers demonstrated that HIV nucleic acid could be directly detected by DNA PCR from the blood of an HIV seropositive person in 1988 (Ou 1988) and shortly thereafter PCR quickly demonstrated its potential in early diagnosis of HIV infected infants (Laure 1988, Edwards 1989, Chadwick 1989, Weintrub 1991). Virological assays have proven to be effective in developed countries and in some resource-limited countries (RLCs). WHO recommends the use of virological tests with a sensitivity of >95% and specificity of >98% for infant and child diagnosis of HIV (WHO 2009).

For settings that lack adequate infrastructure for processing whole blood and cold-chain transportation, DBS offers many advantages. DBS is a reliable source of analyte, which increases access to testing by its ease of collection via heel prick, the lack of cold chain transportation, easily transported from remote areas to more centralized locations for testing, less of a biohazard and stable for long periods of time at ambient temperature (Sherman 2005; Nyambi 1994; Cassol 1991; Cassol 1992; Biggar 1997; Comeau 1996). Collection of DBS has facilitated the detection of HIV-1 in infants as early as 4-6 weeks

after birth (Dunn 1995; Rollins 2002; Sherman 2004; Sherman 2005). The use of properly prepared and maintained DBS permits global participation in the EQA Program. DBS specimens are critical to HIV molecular testing of infants; the qualitative detection of HIV-1 using DBS is the preferred method for EID in resource-limited settings.

With the surge in funding from the U.S. President's Global Health Initiative (GHI) and Emergency Plan For AIDS Relief (PEPFAR), the United Nations Global Fund for AIDS, Tuberculosis, and Malaria (GFATM), The World Bank, The Gates Foundation and other major donors and initiatives, the funding has allowed for the rapid expansion of EID HIV testing to areas in sub-Saharan Africa and other regions of the world previously unable to perform EID testing. To ensure the accuracy of EID of HIV testing as it is being implemented and subsequently scaled up globally with increasing in funding, external quality assurance (EQA) is essential for all testing facilities. A major component of a laboratory's EQA is proficiency testing (PT). Proficiency testing has been shown to improve the quality of testing for various human diseases and analytes (Chalermchan 2007 – Yen-Lieberman 1996). In response to the need for a proficiency test program, the International Laboratory Branch of the Global AIDS Program (GAP-ILB) at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA USA initiated an HIV-1 DNA DBS Proficiency Testing Program. The GAP-ILB program addressed the need for a cost-free proficiency testing program for laboratories performing EID of HIV using DBS cards. This program is voluntary and primarily designed for resource-limited laboratories receiving PEPFAR funds for HIV-related activities.

CHAPTER II - LITERATURE REVIEW

The UNAIDS 2010 Global Report estimates that 33.3 million adults and children living with HIV/AIDS in 2009, an increase from the 2001 estimate of 28.6 million. This increase in people living with the disease can be attributed to increased access antiretroviral drug therapy. Sub-Saharan Africa comprises 22.5 million, 68% of all individuals infected with HIV/AIDS. The annual number of newly infected individuals in this region is 1.8 million, 70% of all newly acquired infections. The number of pediatrics living with HIV globally is 2.5 million. This region saw a decline in newly infected children under 15 from 190,000 in 2004 to 130,000 in 2009. AIDS-related deaths fell from 120,000 to 90,000 during this period, as well. (UNAIDS 2010)

Amplification of proviral HIV-1 DNA in peripheral blood mononuclear cells (PBMC) in seropositive patients using polymerase chain reaction (PCR) allows for a more rapid detection of the virus in three to four days as opposed to viral culture techniques that could take three to four weeks (Ou 1988). The applicability of this technique to detect proviral DNA in infant PBMCs as a rapid and sensitive method to diagnose HIV infection has been demonstrated numerous times (Laure 1988, Edwards 1989, Chadwick 1989 and Weintrub 1991). The 2010 WHO Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access - Recommendations for a Public Health Approach strongly recommends that virologic assays, including nucleic acid tests, be used to diagnose infants less than eighteen months of age and by four to six weeks of age. The most common virologic test used for EID of HIV-1 in RLS is the Roche Amplicor

HIV-1 DNA Test, v1.5 (Roche, Indianapolis IL USA). This test uses PCR to amplify detect HIV-1 proviral DNA sequences in PBMC. The test was designed for whole blood specimens but can be readily modified to be used with dried blood spot (DBS) specimens in a clinical setting. Stevens 2008 reported a consensus between international stakeholders for the use of this test for clinical laboratory testing for the diagnosis of HIV-infected children using DBS in resource-limited settings.

Berk 2005 describes an early antiviral therapy study in HIV-infected infants six to eight weeks of age using CD4 levels as an indicator for initiation of therapy. In this study early antiretroviral treatment (2 months vs 3-4 months) was associated with a delayed and decreased progression of disease. The Children with HIV Early Antiretroviral Therapy (CHER) trial enrolled 377 HIV-infected infants of which 125 infants were randomly selected for deferred antiretroviral treatment and the remaining 252 were randomly selected for early therapy. The CHER study concluded that very early diagnosis and treatment (within two months of age as opposed to three to four months) of HIV-infected reduced infant mortality by 76%. (Violari 2008) A cohort of HIV-infected infants conducted by Obimbo 2004 showed that untreated HIV-infected infants diagnosed before the first two months of life exhibited greater two-year mortality (63%) than those diagnosed later (8%).

The use of dried blood specimens in HIV diagnostics in resource-limited settings allow for collection of clinical specimens in remote areas and transport to an appropriate testing location without the need for maintenance of cold-chain transport. This flexibility in collection and transportation of DBS increases the access to HIV diagnostics for infants. Cassol 1991 first described using this specimen type for DNA detection using PCR on

127 DBS specimens and showed 95.6% sensitivity and 100% specificity when compared to serology results. Cassol 1992 went on to demonstrate the utility of DBS specimens in diagnosing HIV-1 vertical transmission using PCR. Nyambi 1994 Biggar 1997 describes the Malawi Perinatal Intervention Project that evaluated 1,976 duplicate tests on DBS specimens collected from 1,235 infants born to HIV-infected mothers. Results showed a positive PCR results after one month of age was 98.9% accurate in predicting a positive antibody results after 15 months of age. Comeau 1996 Sherman 2005 demonstrated the clinical utility and flexibility of DBS specimens in EID of HIV-1 infection by testing 288 infants born to HIV-infected mothers. Results showed that DBS specimens were 100% sensitive and 99.6% specific when compared to whole blood specimens.

Proficiency testing programs for detection of HIV-1 serve to ensure the quality of results generated by laboratories. Hannon 1989 described a HIV-1 proficiency testing program established by CDC used to ensure the quality of results used in national epidemiologic studies. The program used contrived DBS specimens to establish and maintain external quality assurance of testing facilities used to monitor the HIV seroprevalence of childbearing women. Another CDC program used to assure the quality of facilities that performed HIV antibody testing is the Model Performance Evaluation Program. Schalla 1990 describes the growth of participation in this program in the U.S. from 684 laboratories in 1987 to 1,284 in 1989. Rickman 1993 describes an HIV proficiency testing program sponsored by the Walter Reed Army Institute of Retrovirology that shows improvement in coefficient of variance and standard deviation by participating laboratories performing lymphocyte counting used in HIV patient monitoring.

The AIDS Clinical Trials Group (ACTG) established a proficiency testing program for HIV-1 DNA PCR assays to monitor the performance of laboratories participating in multicenter clinical trials as described by Jackson 1992. This proficiency testing uses 8E5 cells to quantify HIV proviral DNA as developed by Folks 1985. 8E5 cells are PBMCs that have been engineered contain one HIV proviral copy per individual cell. This unique feature allows quantitation of proviral copies using cell counting instruments that are easily accessible. The ACTG also established a proficiency testing program for laboratories providing quantitation of HIV-1 RNA in plasma for clinical trials.

Since 2003 the United States President's Emergency Plan for AIDS Relief (PEPFAR) has provided over \$100B in financial assistance and technical support to resource-limited countries to help stem the global HIV/AIDS epidemic. The first phase (2003-2008) of the PEPFAR program was an emergency response to the epidemic. The second phase of PEPFAR (2008-2013) is aimed at transitioning from an emergency response to a sustainable model for countries receiving support. (PEPFAR website)

CHAPTER III - METHODS AND PROCEDURES

Data Source

Data was collected from laboratories participating in the CDC EQA Program.

Laboratories conducting HIV-1 EID using DBS with PEPFAR-support were informed of the EQA program via email in early 2006 and encouraged to participate. Originally, the 15 PEPFAR Focus countries were encouraged to participate as soon as they developed EID testing capacity. Participating laboratories were assured that their results would be kept confidential. As the CDC EQA Program expanded, all PEPFAR-supported countries were encouraged to enroll and participate. The CDC EQA program also included domestic laboratories performing EID testing that were interested in participating.

Eligibility

Eligibility requirements for inclusion in this study included the following:

Participation in the CDC EQA Program

Ability to perform nucleic acid detection from DBS

Ability to accept correspondence via email

Ability to return test results in a timely manner

Exclusion

Challenge results that were not returned to CDC for evaluation were included in analysis for that time period.

Description of variables

Individual laboratory scores

Results from each challenge were scored from 1-10 dependent on the number of concordance of observed results reported by each laboratory to expected results as determined by CDC. (A score of 10 signified 100% concordance with expected results)

Testing Platforms

Participating laboratories reported using the following testing platforms for the detection of HIV-1 in EID DBS:

Roche Amplicor HIV-1 DNA Test, v1.5

In- house RT-PCR Assay

Roche CAP-CTM HIV-1 DNA Test

Type of laboratory

Laboratories were designated as National Reference Laboratory (NRL), Regional Laboratory (RL), Regional Hospital (RH), or National Hospital (NHO) according to self-reported activities.

Statistical methods

Univariate linear regression was used to show test scores over the observed time period.

Preparation of HIV-1 DNA DBS Proficiency Testing Panel

The DBS panel consisted of 10 blinded DBS specimens: a combination of HIV-1 non-reactive DBS and HIV-1 reactive DBS.

HIV-1 Spiked Whole Blood Preparation

HIV-negative EDTA anti-coagulated whole blood was spiked with a known number of 8E5 cells. The 8E5 cells contain a single integrated copy of the HIV-1 proviral DNA (defective genome) per cell (Folks 1985). Freshly grown or frozen 8E5 cells were counted using a hemocytometer and diluted to varying concentrations (10,000, 5,000, or 1,250 cells/ml) in whole blood.

DBS Card preparation

DBS cards were prepared by transferring 100ul of HIV-negative EDTA anti-coagulated whole blood or HIV-1 spiked whole blood to Whatman 903 filter cards. The HIV-non-reactive and HIV-reactive DBS cards were prepared in separate processing rooms. DBS were dried overnight at room temperature in racks. After drying, the DBS cards were wrapped in glassine paper, stored 10 cards per bag in liquid and gas impermeable plastic sealable bags with desiccant sachets and humidity indicator card and then stored at -20C.

Validation of DBS Cards

The DBS cards were validated by randomly selecting one DBS each from 10 randomly selected DBS cards and excising a 6mm disc from the DBS using a semi-automated hole puncher BSD-600 Duet (BSD Robotics, Australia). Each disc was tested using the Roche Amplicor HIV-1 DNA Test, v1.5 (Roche Diagnostics, Indianapolis IL) according to the Standard Operating Procedures – Amplicor DBS DNA PCR (Guide for Implementation of Services for Early Diagnosis of HIV in Infants in Resource-Limited Settings).

Coding of Proficiency Testing Panel

The HIV-1 DNA DBS panel was blinded for all participants. Each panel contained at least five HIV-reactive specimens and the remaining specimens were HIV-non-reactive DBS specimens. The coding for each panel was randomized from panel to panel.

Preparation of PT Panel

6mm disc PT Panel

Most facilities performing HIV-1 EID used the Roche Amplicor HIV-1 DNA Test, v1.5 with a modification in the nucleic acid extraction step designed for a 6mm disc excised from a DBS specimen. The 6mm discs were excised from the validated DBS cards using the BSD-600 Duet (BSD Robotics, Australia) into 2ml cryovial tubes labeled with the appropriate panel specimen name. The HIV-non-reactive specimens were excised prior to HIV-reactive specimens. Each PT package included the following four items: 1) gas and liquid permeable bag with desiccant sachets and humidity indicator card ten 2ml cryovial tubes each containing a unique 6mm DBS disc, 2) HIV-non-reactive and HIV-

reactive-DBS cards placed in a gas and liquid permeable bag with desiccant sachets and humidity indicator card to be used as quality control specimens for the PT panel testing, 3) testing and reporting instructions, and 4) report form to be faxed, mailed or sent electronically to the GAP-ILB.

Full DBS panel preparation

Facilities using the Roche COBAS Ampliprep/TaqMan System for extraction and detection or the Roche Magnapure Liquid Handler for nucleic acid extraction, requested a full 100ul DBS specimen for testing according to their standard operating procedures. The DBS were excised using scissors and placed inside a 15 ml Falcon tube labeled with the appropriate panel specimen name. The PT package included the following four items: 1) 10 DBS specimens in a gas and liquid permeable bag with desiccant sachets and humidity indicator card, 2) 1-HIV-non-reactive and 1-HIV-reactive-DBS card in a gas and liquid permeable bag with desiccant sachets and humidity indicator card to be used as quality control specimens for the PT panel testing 3) testing and reporting instructions, and 4) report form to be faxed, mailed or sent electronically to the GAP-ILB.

DBS Panel Validation

Two randomly selected panels were tested using the Roche Amplicor HIV-1 DNA Test, v1.5 (Roche Diagnostics) according to the Standard Operating Procedures – Amplicor DBS DNA PCR (Guide for Implementation of Services for Early Diagnosis of HIV in Infants in Resource-Limited Settings)

Shipping

The PT panels were shipped at ambient temperature to the participant facilities via air cargo. CDC DBS Shipping Guidelines were followed.

Results Reporting and Analysis

Participants were expected to report the results of the PT panel testing within four weeks from receipt of shipment. Participants were evaluated on the concordance of their results with expected results validated by CDC-Atlanta. Test scores and peer comparison were returned to the participants via email.

CHAPTER IV - RESULTS

DNA PCR is the preferred test for confirming the diagnosis of HIV infection in infants and young children less than 18 months of age. The lack of access to PCR testing was identified as a major barrier to testing but subsequently overcome with using DBS. DBS can be collected at remote and rural entry sites and transported to a central testing facility. Molecular testing provides an accurate method for identification of HIV-1 but quality testing depends on adequate quality assurance.

Since implementation of the CDC EQA program, 14 panels have been shipped including twice in 2006 and tri-annually in 2007, 2008, 2009 and 2010. Initially, CDC Program began shipping specimens to 17 EID testing facilities in 11 PEPFAR-supported countries and membership has steadily increased over time to include greater than 33 countries and 100 EID testing laboratories (Figure 1).

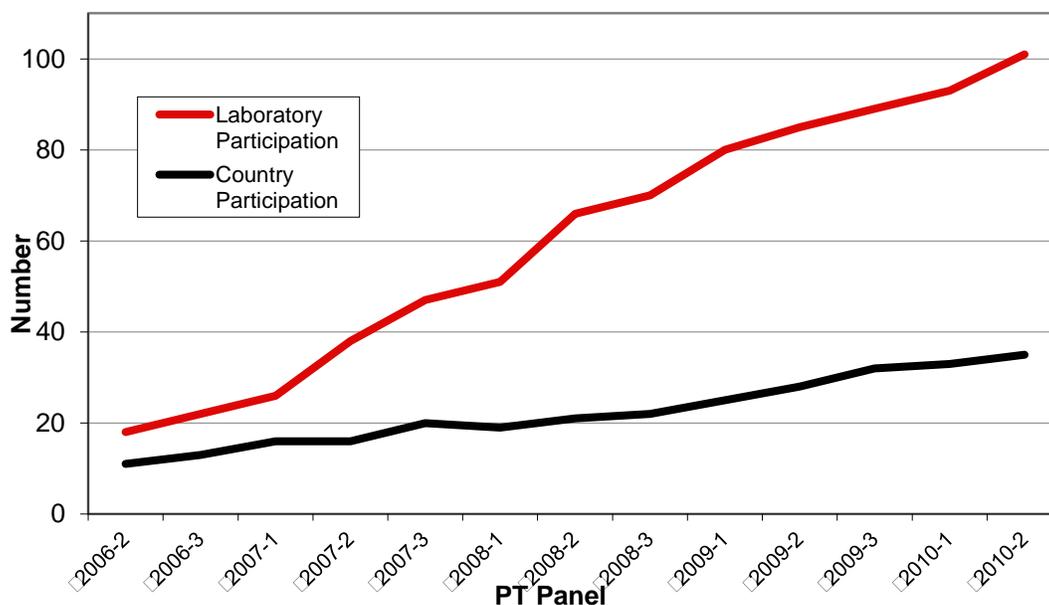


Figure 1: Number of laboratories and countries enrolled at each panel distribution time point

The Program expansion has shown an increase in the number of laboratories from 17, 25, 51, 76, and 95 in 2006, 2007, 2008, 2009 and 2010 respectively. The global distribution of participating facilities in 2010 was as follows: 68 laboratories from 18 sub-Saharan African countries, 6 labs from 3 Southeast Asia countries, 2 labs from Caribbean region and 1 North American, South America, Asia and Oceania laboratory each.

Within the last several years, many successful EID programs have been established in sub-Saharan Africa and other high burden countries, and there is a combination of types of laboratory testing facilities conducting the molecular testing and participating in the CDC EQA Program. As expected, the majority are either national reference laboratories (NRL) (n=28) or regional reference laboratories (RRL) (n=24). Two additional facilities were identified as both NRL and regional hospitals (RH). Twenty facilities serve as both

RRL and RH, 8 facilities were identified as RH and another two as National Hospitals (NHO) (Figure 2).

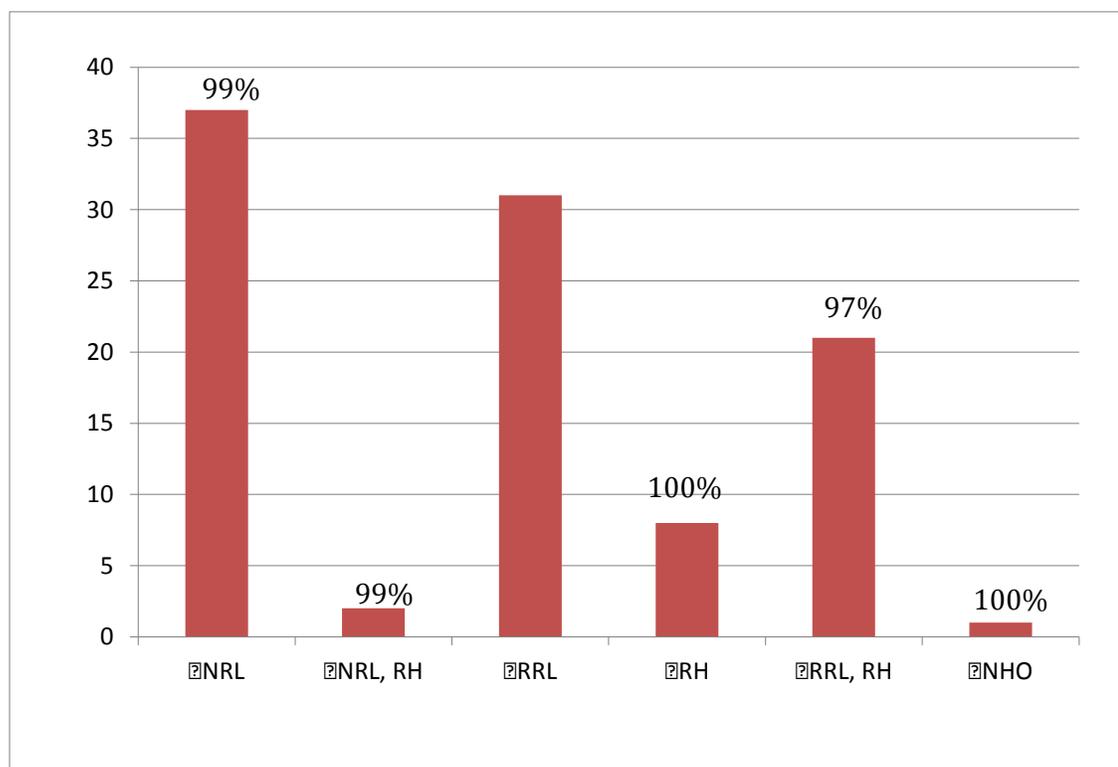


Figure 2: Categories of participating laboratories conducting DNA PCR for EID

NRL = National Reference Laboratory; RRL = Regional Reference Laboratory; RH = Regional Hospital; NHO = National Hospital

DBS specimen proficiency testing panels were sent tri-annually to participating laboratories for them to test and return results reports within a four-week period. Scores were then returned to the laboratories in a timely manner to provide feedback on how well their facility performed on the panel. An overwhelming majority of the participating laboratories (89.7%) returned the results electronically to CDC-Atlanta at each time point with a range of 76.5 - 100% (data not shown). As a measure of testing performance over time, the mean scores (number of correct results out of 10) were determined for each panel (Figure 3).

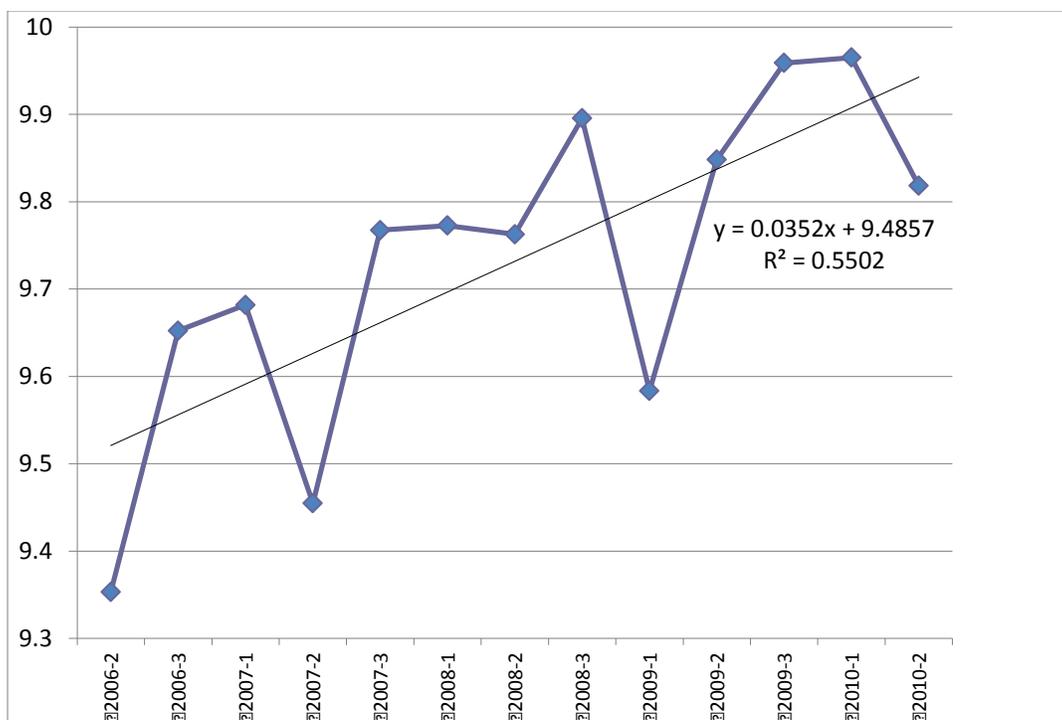


Figure 3: Mean Score of all laboratories over time

The mean range was determined to be 9.35 – 9.96. The lowest mean score (9.35) was observed in the original PT panel and the highest mean score (9.96) was recorded in the third PT panel of 2009. There is a positively increasing trend ($y = 0.0396x + 9.4639$, $R^2 = 0.507$) in mean scores over time.

In 2006, the Roche Amplicor HIV-1 DNA Test, v1.5 (Roche, Indianapolis IL USA) was recommended for laboratory testing for the diagnosis of HIV-infected children less than 18 months of age (Stevens 2008) and it is the most widely-used testing assay reported in the HIV-1 EID PT Program (Table 1).

Table 1: Testing Platforms

	Roche Amplicor HIV-1 DNA Test, v1.5		In-house developed-Real-time		Roche CAP-CTM	
	Number of laboratories performing assay	Average score	Number of laboratories performing assay	Average score	Number of laboratories performing assay	Average score
2006-2	17	9.3				
2006-3	23	9.6				
2007-1	22	9.7				
2007-2	33	9.5				
2007-3	42	9.8	1	10		
2008-1	38	9.8	1	10		
2008-2	55	9.7	3	10	1	10
2008-3	64	9.9	2	10	1	10
2009-1	66	9.6	3	10	3	10
2009-2	72	9.9	5	9.6	4	9.7
2009-3	62	10.0	5	10	6	10
2010-1	68	9.9	6	10	13	9.9
2010-2	60	10	9	9.8	17	9.6
2010-3						

Some laboratories use various in-house-developed real-time PCR assays for analyzing the PT specimens. Four of six laboratories using the Roche COBAS Ampliprep/Taqman (Roche, Indianapolis IL USA) system previously reported using the Roche Amplicor HIV-1 DNA Test v1.5 (Roche, Indianapolis IL USA) (Table 1). Encouragingly, despite the move to other testing platforms including the slight increase in in-house assays, the mean PT results are greater than 9.5.

The longer laboratories participate in the CDC EQA Program, it is expected that the mean results should increase over time. The laboratories were categorized into number of months participating in the PT program and there was no decay in the ability to score well (Figure 4).

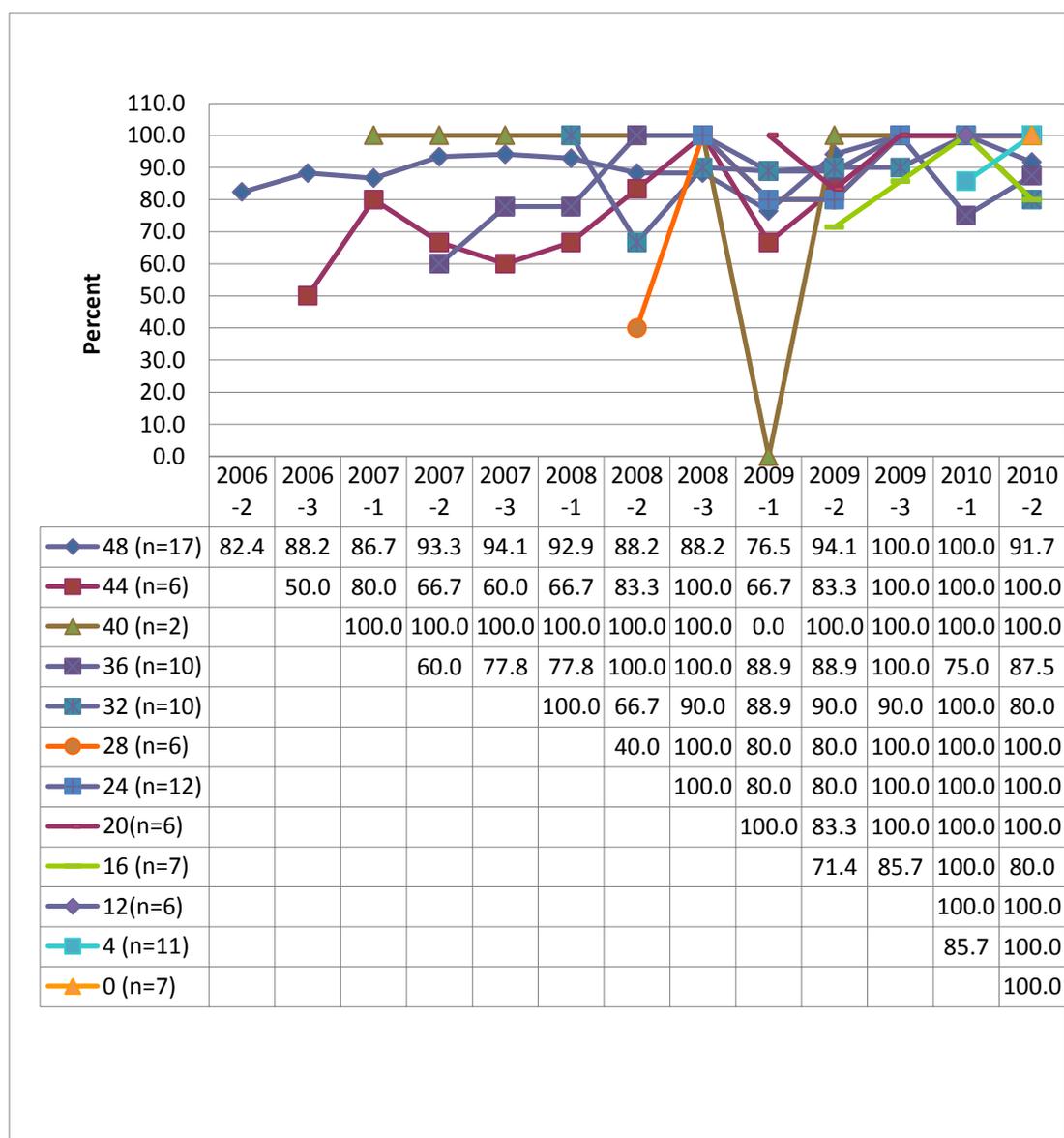


Figure 4: Percentage of laboratories scoring 100% at each time point based on number of months participating in the PT Program

In fact, the trend was for testing to improve over time. There was an anomaly in 2009-A where 4 testing labs reported results with mean scores of 7 or less. In fact, two labs reported results of 4 of 10 correct results (data not shown).

Laboratory testing is a highly complex process, which is typically divided into three main phases (pre-, intra- and post-analytical). In the analytical phase, the Roche Amplicor HIV-1 test consists of greater than 40 individual steps that could result in an inaccurate finding. However, a mark of success is the decreasing level of errors in the analytic phase with the high level of accuracy that currently exists in DNA PCR. Over the course of the program we observed missed tests on every panel, including either false negative and false positive findings or equivocal results. Except for the original testing where the majority of discordant results were false negative, the range of reported misses was typically 0 - 2 % (Figure 5.)

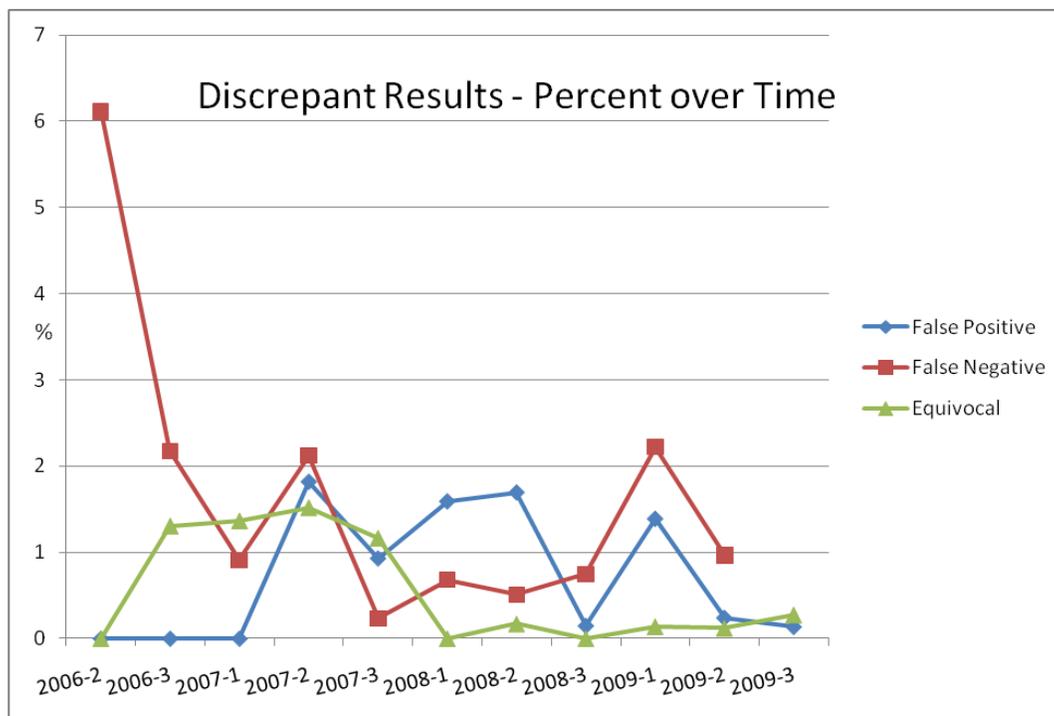


Figure 5: Total percent of discordant results by all laboratories. Discordant results are false positive, false negative and equivocal (OD value between >0.2<0.8)

Laboratories were twice as likely to report false negative than false positive results (1.67% vs 0.72%).

Twenty-three countries participating in the EID PT Program have either one or two testing laboratories while only 12 countries have more than 3 or more EID testing labs. We observed that as several countries scaled-up and added testing facilities conducting DNA PCR to accommodate primarily logistical needs, there is an overall trend for lower quality results (Figure 6).

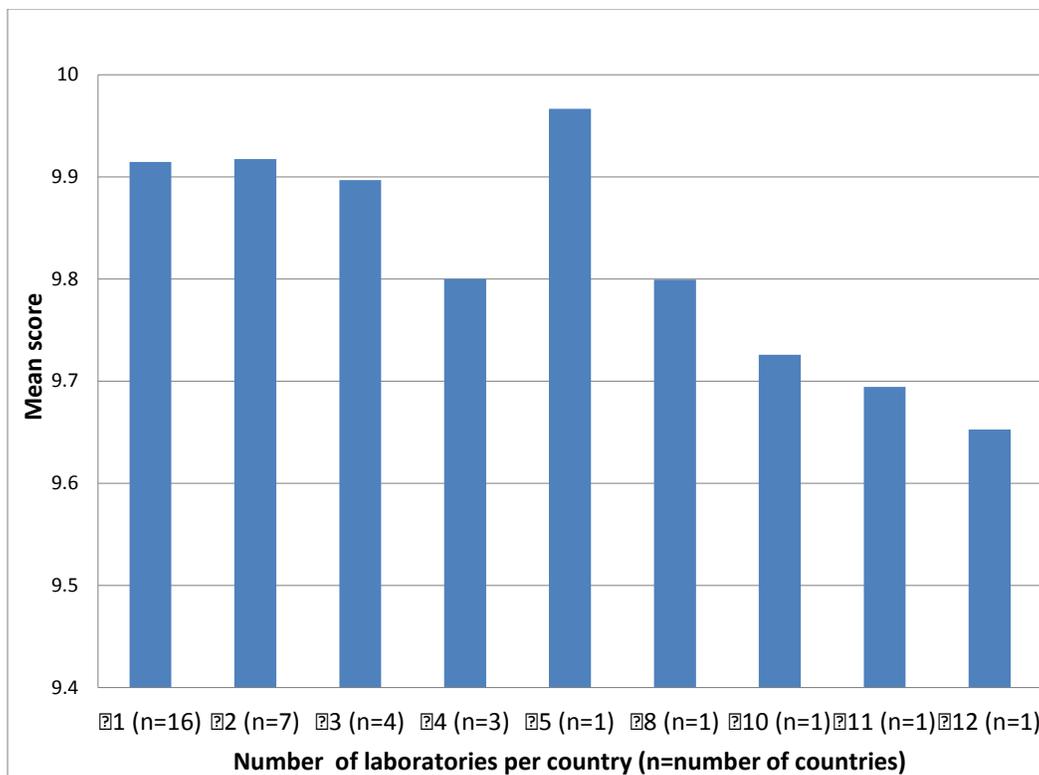


Figure 6: Mean scores of laboratories according to number of laboratories per country

CHAPTER V - DISCUSSION AND CONCLUSION

As EID testing began rapidly expanding worldwide in the past decade, there was an urgent need for an external quality assurance program to monitor the quality of HIV DNA PCR testing on DBS. An innovative but simple approach was developed to help laboratories monitor the quality of PCR testing for EID in resource-limited settings. The CDC-Global AIDS Program embarked on a distinctive path to globally accelerate improved management and good laboratory practice of EID testing by DNA PCR. The CDC established an EQA program in 2006 and has expanded it over the past three years to include laboratories on 4 different continents including Africa, Asia, North and South America as well as Latin America, Caribbean Region and Oceania. The cost of the operation is covered by the PEPFAR and consists of 4 components: 1) provision of proficiency testing panel, 2) provision of known positive and negative DBS specimens to be used as internal controls, and 3) summarize PT results for end users, and 4) provision of technical assistance or remedial intervention measures.

Advances by resource-challenged countries to conduct EID in infants have resulted in more children being tested. To meet the burden of high volume EID testing, several sub-Saharan African countries have transitioned to using an automated extraction platform such as Roche COBAS Ampliprep-COBAS TaqMan. With this switch to automation, there are expectations that analytical stage errors will be reduced because in recent decades, standardization, automation and technological advances have significantly improved the analytical reliability of laboratory results and decreased the error rates

(Stankovic 42). However, there is always room for improvement, particularly in areas such as molecular assays, and for more effective procedures for quality assessment and control. The routine use of quality control specimens as part of quality assurance program prompted CDC to provide known positive and negative DBS specimens with each proficiency test panel. Based on the projected needs of the laboratory, CDC provides more than an adequate number of DBS internal controls to be used with each testing run until the next panel shipment.

Implementing a comprehensive laboratory quality management system and following good laboratory practices can reduce the frequency of testing errors associated with molecular diagnostic testing such as DNA or RNA PCR that characteristically involves multistep processes. The variability of test results and the frequency of errors can be reduced with a dedicated comprehensive quality management system. This typically includes at minimum site supervision, retesting of specimens and participation in routine proficiency testing, all key components of laboratory accreditation. Proficiency testing is a widely used approach to monitor the quality of testing and can result in an increased accuracy in results and increased confidence by health care providers (Glencross 2008). For example, participation in an external CD4 proficiency test program (Canadian QASI-Quality Management System) resulted in the reduction in errors by 26-38% in simply 3 rounds of testing in resource-limited settings (Bergeron 2010).

Very few clinical laboratories in sub-Saharan Africa have earned internationally recognized accreditation for testing of clinical specimens (Peter 2010) and this includes most of the PT participating laboratories. The impressive increase in laboratory participation and number of countries demonstrates the need for a cost-free EQA

Program for laboratories performing HIV EID in resource-limited settings. Many of the PEPFAR-supported laboratories, which are struggling with inadequate resources, are encouraged to continually improve for a standardization of practices and seek accreditation from the new WHO-AFRO Program for Quality Improvement that is supported by PEPFAR (Gerhsy-Damet 2010). This accreditation program uses a novel five-step process to allow labs to gradually move towards accreditation in a cost-effective manner that positively reinforces progress at each step. One of the requirements for this accreditation is participation in an EQA Program for each assay used in patient diagnostics and monitoring. Laboratory accreditation will help strengthen the laboratory and health system in these countries burdened with disease.

Before enrollment into appropriate care and treatment programs, too many HIV-infected infants and children are either never diagnosed properly or disappear from the healthcare system. A poor quality DNA PCR test combined with a long turn-around time could mean a useless result. Therefore, the CDC EQA Program provides the blinded DBS panel and requests that laboratories treat the PT DBS as if they were clinical samples and return the results electronically (fax or email) to CDC-Atlanta within four weeks to mimic the 30 day turnaround recommended by WHO (WHO reference). Unfortunately, over time consistently greater than 10% of the laboratories failed to return the results within the expected time frame. The laboratory test itself is only a minor component in an EID program, which typically includes proper specimen collection, DBS transport to the lab from the clinic, DNA PCR and result return, and this entire process should occur within 30 days. To not meet the four-week turnaround may indicate a focal point for strengthening good laboratory practices. There are several obstacles to returning reliable

results in a timely manner for many of these participating labs; lack of infrastructure in some of these countries causes problems as well as specimen transportation delays, inconsistent power sources, lack of skilled workers, reagent procurement delays, stock outs, insufficient training and low wages for skilled workers. All of these issues were reported as reasons for not providing EQA test results in a timely manner and these factors as well as insufficient education may lead to producing more unacceptable results (Delost 2009) and lead to a decrease in the quality of testing being performed in these facilities and the reliability of the results returned to patients.

Laboratories that achieved less than 80% concordance with the expected results were contacted and most participants who responded were able to troubleshoot their issues. A vast majority of the discordant values were attributed to transcriptional errors, post-PCR contamination, wrong test performed and/or improper results interpretation, which are very much in alignment with Plebani (2006) where frequent causes for failure in proficiency testing are random human errors to errors caused by faulty testing material. Despite years of quality management in the United States, errors in the analytical stage are estimated to range between 7 - 12% (Plebani 2006, Kalra 2004), stressing the importance of good laboratory practice in resource-rich and resource-limited settings.

The upper limit on the total discordant results was generally between less than 4% for any testing panel with the exception of the very first PT panel; there was a higher rate of false negative results at time 2006-1 compared to the remaining time points. The elevated level of false negative results was likely attributable to the PT specimens (6mm DBS punches) containing a relatively low DNA copy number (1,250 copies/ml). The 2006-1 observation was primarily confined to laboratories using the Roche MagnaPure Liquid

Handler (Roche Diagnostics, Indianapolis IL) for nucleic acid extraction in conjunction with the Roche Amplicor assay. Discrepancies between manual nucleic acid extraction and Magna Pure LC have been reported (Schuurman 2005 and Patton et al 2007). The laboratories using the MagNA Pure extraction of DNA evaluated whole DBS specimens (13mm punches) from that time point forward and there was no indication of decreased sensitivity from those laboratories after the adjustment to specimen volume was made.

With the increased demand for proficiency testing in EID and imminent unveiling of point of care of near-care diagnostic molecular devices, the next logical step is to transfer the PT DBS technology to the resource-limited settings. Through this technology transfer many of the logistical difficulties in panel distribution and transportation delays due to problems with customs authorities will be overcome. With an in-country panel production, countries must develop strong external quality assurance programs to support the increase in testing demand and testing facilities. As is observed in Figure 6, which displays a trending of decreasing PT scores with increasing number of labs per country, this trend strengthens the argument that laboratories performing EID should participate in a robust in-country laboratory network with EQA to monitor their performance and evaluate themselves among other peers. It also demonstrates a further need for countrywide monitoring of EID programs during the impending scale-up phase of growth. Furthermore, PT programs are quite effective as an EQA tool in identifying poorly performing test sites (Peddecord 1992).

The public health impact of the CDC EQA program may be observed over time as EID programs become more widespread in resource-limited settings. Increased quality EID testing has the potential to increase the efficacy of PMTCT programs by improving the

accuracy of diagnostic results. Improved diagnostic results can be used as indicators of efficacy for PMTCT programs giving program coordinators and other major stakeholders a more accurate representation of their program's success. Increasing the quality of the EID activities in a laboratory will hopefully result in an increase in quality in other molecular testing activities by instilling better procedures. As a result the quality of molecular diagnostic testing in the resource-limited settings where the CDC EQA Program is implemented will hopefully increase over time as a result of participation.

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