ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Evaluation of the DBS versus plasma sample with HCV testing using ELISA and RDT diagnostic methods at Ethiopian National Blood Bank Service, St.Peter Hospital, Yekatit 12 Hospital, CBE Clinic and St. Paulo's Hospital Millennium Medical College Addis Ababa, Ethiopia.

By: Mulukan Akalu (BSc, MSc candidate)

Advisors: Dr. Mistire Wolde(MSc, Ph.D. and associate proffesor)

Ms. Sossina Walelign (MSc)

Mr. Atsebeha G/Egzihabher(MSc, PhD candidate)

A Research thesis submitted to the Department of Medical Laboratory Science; College of Health Science, Addis Ababa University, in partial fulfillment of Master of science Degree in clinical laboratory science (Diagnostic and Public Health Microbiology)

June, 2023

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Mulukan Akalu, entitled: Evaluation of the DBS versus plasma sample with HCV test results done by using ELISA and RDT diagnostic methods at Ethiopian National Blood Bank Service St.Peter Hospital, Yekatit 12 Hospital, CBE Clinic, and St. Paulo's Hospital Millennium Medical College, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner Dr.Zewdu Terefework Signature  Date 27 June 2023

Internal Examiner _____ Signature _____ Date_____

Advisor _____ Signature _____ Date_____

Advisor_____ Signature _____ Date_____

Chairman of the Department or Graduate program coordinator

_____ Signature _____Date_____

ACKNOWLEDGMENTS

First of all, I am grateful to the Almighty God for without his graces and blessings writing this research thesis would not have been possible. I would like to express my gratitude to my advisors, Dr. Mistire Wolde, Ms. Sosina Haile, and Mr. Atsebeha G/Egziabher for their support, patience, and encouragement while writingthis research thesis. I would like to thank Addis AbabaUniversity, College of Health Sciences, Department of Medical Laboratory Sceinces , Ethiopian Nation Blood Bank (NBB), St.Peter Hospital, Yekatit 12 Hospital, CBE Clinic , St. Paulo's Hospital Millennium Medical College and Ethiopian public health institute for set up and supplies, to conduct my thesis.

My grateful gratitude goes to all study participants and data collectors for their support to get appropriate data.

Last but not least, I wish to express my deepest gratitude to my families and friends for their help, kind and everyday support in my life

Table of Contents

List of Tables

Abbreviations

AAU	Addis Ababa University
Ab	Anti-body
CAP	College of American Pathology
CBE	Comerial Bank of Ethiopia
CHS	College of Health Science
CI	Confidence Interval
DBS	Dry Blood Spot
DOR	Diagnostic Odds Ratio
EIAs	Enzyme Immunoassays
HCV	Hepatitis C Virus
NBTS	National Blood Transfusion Services
NLR	Negative Likelihood Ratio
NPV	Negative Predictive Value
PLR	Positive Likelihood Ratio
PPV	Positive Predictive Value
RDT	Rapid Diagnostic Test
RIQAS	Randox International Quality Assessment Scheme
SROC	Summary Receiver Operating Characteristic
WHO	World Health Organization

Abstract

Back ground: One of the numerous diseases that the HCV can cause is cancer. It is a significant public health issue that has a global impact on millions of people's lives, with majority from sub-saharan African countries. Laboratory diagnosis of HCV mainly done by using ELISA, and Rapid diagnostic test methods by using plasma/serum samples. However, most laboratories do not have the facilities & so forced either to submit patients in the far referral laboratories or empirically treat patients. Thus, as alternative solutions, other places worldwide used dry blood sample techniques to collect sample & submit for laboratory tests. However, such DBS approaches not well known in our country.

Objective: To evaluate the DBS versus plasma sample with HCV testing using ELISA and RDT diagnostic methods at Ethiopian National Blood Bank, St.Peter Hospital, Yekatit 12 Hospital, CBE Clinic, and St. Paulo's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Methods: Cross-sectional investigation were used to detect the effectiveness of DBS using whole blood in the (ELISA-INNOTEST hepatitis virus Ab IV) compared to the serum/plasma sample with RDT (wondfo) tests for the detection of HCV antibody . Data was collected by a check list. 159 HCV negative and 159 HCV positive venous plasma samples and 159 HCV negative and 159 HCV positive whole blood was tested with the standard INNOTEST HCV Ab IV assay and RDT(Rapid diagnostic test).

Results: A total of 318 individuals were participated. Participants age range of the study were 18 to 62, whereas, the DBS sample HCV test result using ELISA & RDT tests were 159 positive,159 negative and 156 positive,162 negative respectively. The sensitivity, specificity, PPV, NPV and efficacy of DBS versus ELSA& RDT were 100%, 99.4%, 99.4%, 100%, 98.7%, 99.4%, 98.7%, 99.4%. The test result correlation agreement between of DBS among ELSA & DBS-RDT was 0. 969 & 0. 963 respectively.

Conclusions recommendation: The INNOTEST® HCV Ab IV assay performance using DBS was acceptable, WHO recommend that, sensitivity for detection of antibodies to HCV was 98% and specificity 99%, thus DBS may be used as a substitute sample type in cases when venous blood collection is not feasible.

Key words: DBS, HCV, sensitivity, specificity, PPV and NPV

1. Introduction

1.1 Background

A variety of malignancies presented with HCV, including hepatocellular carcinoma and lymphoma[1]. It affects between 130 to 150 million people worldwide[2] with 80% of them reside in countries with low and moderate incomes[3]. 3.1% of Anti-hepatitis C virus is present in Ethiopians[4,5]. Prisoners, sex workers, the homeless, and other institutionalized people are among the population groups who are more likely to contract HCV than others[6,7].

The HCV can cause both chronic and acute hepatitis, with signs and symptoms from a minor disease to a critical, such as cirrhosis and cancer of the liver. Because HCV is a blood-borne virus, the majority of infections develop as a result of unsafe injection procedures of blood, injectable drug usage, sexual acts and unscreened blood transfusions that expose persons with blood. Because HCV infection is silent until decades after infection, when symptoms arise as a result of severe liver damage[1].

Hepatitis C infection must be diagnosed in order to begin treatment and avoid transmission. For the WHO's (World Health Organization) eradication goals to be successful, expanding HCV testing is required for monitoring HCV- antibodies. The quality control result of RDT was challenging in addition with their simplicity and convenient to use, and this result make it difficult to acquire data for prevalence controlling. Analyzing in referral laboratory implementing EIAs with quality assurance solve the problems. Insufficient medical equipment in resource limited nations makes difficult to process sample with EIA. [1,8].

To days laboratory analysis lowers HCV detection in area with laboratory instrument is difficult to accessible, as well as in many regions of the world where diagnostic equipment is inadequate. This makes HCV diagnosis problematic in areas with few resources. These challenges indicate the requirement for alternative anti-HCV diagnostic procedure that requires a little amount of blood and is widely available for HCV infection identification. As a result, DBS are a frequent less invasive sampling approach that simplifies sample collection and storage[9].

DBS provide several advantages over typical serum, plasma, and blood collection methods. Dried spots on filter paper are simple to draw and perform, need few amount, no refrigeration needed and transported with little risk of biohazard. In addition, Nucleic acids, antibodies,

numerous medicines and their metabolites stable on DBS card for longer than in routine samples when kept at room temperature. These benefits allow a lot of flexibility in terms of sample collecting and transportation techniques[10].

In 2017, the World Health Organization (WHO) advised that DBS samples be used to improve HCV testing accessibility. Whole blood is added to DBS card and after dried it was delivered to referral laboratory, eluted and examined. DBS allows for the storage of samples at room temperature. DBS gives a practical, affordable alternative for sample collection in resource limited nations and in remote setting with little resources and no access to direct testing for HCV or medical service.[11,12]. DBS can withstand prolonged exposure to normal temperatures, has little risk of biohazard, and is simply sent via postal services to a central laboratory. [13,14,15]. DBS can reduce cost and time to diagnose HCV for individuals who are positive for anti-HCV and used for RNA confirmatory testing [16].

Using a EIA, detection of anti-HCV antibodies in whole blood, followed by a molecular confirmatory test in case of positivity, is commonly used to screen for HCV infection[16 ,17]. Whole blood is used to detect viral hepatitis. In persons of all ages, however, DBS is an effective substitute than serum. According to WHO guidelines, the use of DBS is a priority activity for HCV testing. However, the p effectiveness and viability of DBS for the detection of Hepatitis C in several resource limited nations in Sub-Saharan Africa, such as Ethiopia, has yet to be reported. These studies highlight the requirement for careful assessment of DBS performance[18].

1.2 Statement of the Problem

Around 71 million people are infected with the chronic hepatitis C virus worldwide, with an anticipated 50,000 new infections annually in 2018[13,19]. HCV infection is predicted to affect 27 million persons in Africa with 0.9 to 6.0% as the regional prevalence percentages. In Ethiopia, there are 3.1% anti-hepatitis C virus (anti-HCV) antibodies[20]. During initiation of infection there was no symptom, and access to diagnostic testing is limited due to financial and logistical constraints[19].

Expensiveness and limited availability of HCV detection tests, access to conventional assays is difficult for patients infected with HCV, especially for individuals who live in remote areas[1,21]. These issues can be addressed by testing using enzyme immune assay (EIAs) in referral laboratories. For EIA testing serum or plasma sample collection might be challenging, due to human power, laboratory equipment's and temperature control for transportation in resource limited nations [13,16,22].

Dried blood spot was important for detection of HCV patients living with resource-constrained environments, which makes it crucial to assess its efficacy for hepatitis C testing. DBS is effective in testing for hepatitis C as confirmed in numerous studies. The uncertainties are a result of lack of governmental permission to have dried blood spot and the manufacturers' survey analysis procedures for using DBS for anti-HCV testing. When human power, laboratory equipment's and temperature control for transportation is challenging in low and middle income countries, only empirical data can determine whether DBS samples may be used in place of blood samples to find anti-HCV antibodies. It is thus reasonable to conduct a sensitivity and specificity analysis of DBS for HCV antibody detection[8,13,16,22]. Need of this investigation to assess DBS as an appropriate substitute sample type by comparing its performance with ELISA.

1.3. Significance of the study

DBS provide several advantages over typical serum, plasma, and blood collection methods. Dried spots on filter paper are simple to draw and perform, need only a few amount, no refrigeration needed and transported with little risk of biohazard. In addition, Nucleic acids, antibodies, numerous medicines and their metabolites are stable on DBS card for longer than in routine samples when kept at room temperature. These benefits allow a lot of flexibility in terms of sample collecting and transportation techniques and to solve the challenges the survey systems have faced.

The findings of this study also aid medical professionals in the detection and treatment of HCV. This is important for other researcher and basic for investigator.

Once more, the study aids medical professionals and other service providers in accurately identifying HCV-infected people for treat and care. When individuals are tested, they are informed of their infection status, enabling them to take action to prevent the progression of HCV-associated disease and lower their risk of spreading the virus to others.

2. Literature review

This study focus on evaluation of DBS sample for the detection of HCV antibody. DBS is performed by adding a drop of whole blood on a prepared DBS card and another type of sample to detect HCV antibody. To explore scientific literature, the Google scholar and pub med software data base is used.

A cross-sectional study conducted; on 108 patients infected with HCV-were used to evaluate DBS for hepatitis C virus screening. 100 positive DBS samples and 8 falsely negative DBS samples were observed, with 92.6% sensitivity. The INNO-LIATM HCV Score test was used to retest the 8 false negatives, and it produced 4 positive samples, 3 negative samples, and 1 indeterminate result. Additionally, HCV antibodies were not present in 31 DBS samples from the 31 people who tested negative for HCV, with 100% specificity. Furthermore, the NLR was 0.07, the NPV was 79.5%, and the PPV was 100%. No false positive result, therefore PLR were not determined [8].

The Study done on prospective analysis of the hepatitis C virus antibody detection in whole blood gathered on DBS with the INNOTEST® HCV Ab IV enzyme immunoassay. In contrast to plasma as the benchmark, the INNOTEST® HCV Ab IV assay had 100% sensitivity and specificity for finger prick DBS or venous blood DBS. 100% agreement between all sample types[8,9].

Two systematic reviews and meta-analysis studies conducted on detection capacity of hepatitis C serological diagnosis having DBS samples reported that, a narrative evaluation for detecting efficacy of HCV-Ab contained data from 23 research, while a pooled quantitative meta-analysis included data from 19 investigations. Specificity and sensitivity were 99% (CI95%: 98–100) and 98% (CI95%: 95–99), respectively. In both systematic reviews, general quality of studies and heterogeneity has been described as moderate [13].

Based on the comparative study on anti-HCV testing using plasma, dried plasma spots (DPS), and dried blood spots (DBS) done in china, from 329 persons were tested, using plasma results as the benchmark, samples were tested by ELISA for anti-HCV and the capacity of DPS and DBS sample were tested. According to the plasma result, 118 samples (59.0%) tested positive for HCV. Therefore for detection of HCV the sensitivity, specificity and kappa value of DBS and DPS result were 98.3%,100%,98.7% and 99.2%,100%,99.3% respectively. In comparing plasma

among DPS and DBS sample result and DPS and DBS sample showed Spearman's correlation coefficients of 0.857, 0.750, and 0.739, respectively. In comparison to the plasma results, DBS and DPS sample report negative for 2 positive samples and not detected for one sample respectively. DBS has a potential replacements for plasma to detect anti- HCV[11,23].

Study conducted in France; DBS: used for detection, diagnosis and treatment monitoring for HCV. 511 people in total were included, 170 subjects were HCV-sero negative and 341 patients were HCV sero-positive. All DBS were evaluated for the presence of total anti-HCV antibodies. It was associated with 99.1% sensitivity and 98.2% specificity[24].

According to a study conducted in Malaysia on the detection anti HCV assay, the detection of Hepatitis C virus antibodies (anti-HCV) utilizing DBS samples had a good level of accuracy, with an ideal cut-off value of 0.10. DBS have 97.3% and 100% sensitivity and specificity respectively[25].

A Systematic review and meta-analysis studies conducted in Congo on detecting capacity of DBS for hepatitis C antibodies indicates, twelve of the eleven examined using enzyme immunoassays (EIAs) to analyze the anti-HCV, and the rest employed quick analytic tests. The majority of the research (83.3%) was case-control studies from industrialized nations (66.7%). The capacity of dried blood spot to detect HCV has 98.1% sensitivity and 99.7% specificity. The pooled sensitivity and specificity in investigations using EIAs were 97.3% and 99.6% respectively [12].

A scientific report from Cameroon compared the performance of Abbott ARCHITECT anti-HCV analyzer using venous blood samples Dry blood spot to the conventional assay using venous plasma and DBS samples. A retrospective investigation involving convenience sampling in Cameroon yielded a positive 144 HCV and 104 negative HCV compared to plasma and venous blood samples. The results of the modified volume DBS assay (R2 = 0.71 and 0.99, respectively) were strongly correlated with the standard assay result done by plasma on dilution series and patient samples. DBS has increase the ability to detect HCV for individuals that may not have direct access to HCV care[21].

The study in Cameroon reports sensitivity, specificity, PPV, NPV of DBS were 99%, 98%, 98%, 99% respectively and 0.97 kappa index. This study shows that DBS was alternate specimen type for the diagnosis of HCV[22].

Study conducted in Burkina Faso; combining rapid diagnosis and DBS assay for point of care examination HIV,HBV and HCV shows among 213 total tests 5 positive and negative plasma sample for HCV were 5 positive and negative by DBS, sensitivity, specificity, PPV, NPV were 100% and kappa value1.0 [26]

3. Objectives

3.1 General objectives

Assessment of DBS detection capacity versus plasma sample with HCV test results done by using ELISA and RDT diagnostic methods for detection of HCV– Antibody at Ethiopian National Blood Bank service, Addis Ababa, Ethiopia.

3.2 specific objectives

- To compare DBS sample associations with plasma sample using ELISA and RDT diagnostic method for the detection of HCV antibody at Ethiopian National Blood Bank service, Addis Ababa, Ethiopia.
- To determine the DBS samples' sensitivity and specificity with plasma sample using ELISA and RDT detection of anti -HCV at Ethiopian National Blood Bank service, Addis Ababa, Ethiopia.

4. Materials and Methods

4.1. Study area

The investigations done at Ethiopian National Blood Bank Services, St. Peter Hospital, Yekatit 12 Hospital medical college, Commercial Bank of Ethiopia clinic and St. Paulus Millennium Medical College the place were sample collected and transported to EPHI for laboratory analysis.

Teaching and promotion are the main duties of Ethiopia's National Blood Bank Services Office, which is a non-profit governmental organization. The Ethiopian Red Cross society established the National Blood Transfusion Services (NBTS) in 1969. In 2004, teaching, promoting and laboratory blood services in Ethiopia were under control of ministry of health. Its primary headquarters are in Addis Ababa, around Goma kuteba and it is also responsible for overseeing, supporting, and monitoring the service of regional blood banks across nationally, which was administered by regional health bureaus.

St. Peter's Specialized Hospital is located in Gullele sub-city, Addis Ababa, Ethiopia and established in 1960 GC. The hospital is a TB Specialized Hospital with more than 300 beds and gives different inpatient and outpatient services including HIV counseling and testing, and ART .

Yekatit 12 Hospital medical college. It is found near sidist kilo, in Arada Sub-City of City Government of Addis Ababa. It was established in 1915 and its former name is Kedamawi Haile Selassie. Hospital started to work with a total of 25 beds and 37 health professionals with few facilities and reached today level, the hospital has 912 health professionals and 372 administrative staff with around 370 beds and compromising many departments which gives different services like screening and diagnostics, specialized procedure, specialty and sub specialty clinics. It is serving more than 5 million people in the catchment area. The hospital accepts patient"s through referral system and private wing.

St. Pawulos Miliniem Medical College is located in Addis Ababa, Ethiopia and which has 650 beds and an annual average population of 700,000. It was established by Emperor Haile Selassie I in 1969 with support from the German Evangelical Church. It's catchment area includes more than 5 million people. There are different departments in St. Paul Hospital Millennium Medical

College, including forensic Medicine, Toxicology, Internal medicine, Neurology, General surgery, Psychiatry, Ophthalmology, Dentistry (maxillofacial surgery), Radiology, Dermatology, Gynecology, Obstetrics, Pediatrics, Emergency medicine, Neurosurgery, pharmacy, radiology and Medical Laboratory Department.

4.2. Study design and Period

Transverse investigations were carried out, from June 2022 - March 2023.

4.3 Population

4.3.1 Source population

Whole blood samples used to screen for blood transfusion at Ethiopian National Blood Bank Services and for liver disease St. Peter Hospital, Yekatit 12 Hospital medical college, Commercial Bank of Ethiopia clinic and St. Paulus Millennium Medical College.

4.3.2. Study Population

Left over samples those screened for HCV at the time of study and fulfills the requirements.

4.4. Inclusion and Exclusion criteria

4.4.1. Inclusion criteria

Samples with the acceptable criteria based on the laboratory SOP including with proper specimen collection, with proper volume of blood ratio to anticoagulant, and non-clotted sample was added within investigation.

4.4.2 Exclusion criteria

A Blood sample that has hemolysis, lipemic, and clotting and generally that do not fulfill the laboratory SOP was omitted from investigation.

4.5 Study variables

4.5.1 Dependent variables

- Performance of ELISA and RDT using DBS samples to diagnostic HCV.
- Sensitivity, specificity, PPV and NPV.
- Age and Sex.

4.5.2. Independent variables

- Performance of ELISA and RDT using serum sample diagnostic HCV.

4.6 Sample Size Calculation and Sampling Methods

4.6.1. Sample size determination

For evaluation of DBS for HCV detection, Clinical and Laboratory Standard Institute (CLSI) suggested 40 to 100 samples were applied to evaluate two approaches – Antibody; the CLSI EP09 guideline was followed. In line with CLSI EP09-A3 (Measurement method evaluation with error calculation regarding human specimen), for our investigation 318 specimens were used which are 159 seropositive and 159 negatives HCV[27].

4.6.2. Sampling method

Until the sample size was reached, Study participants who fulfilled with the inclusion criteria were included using a practical sampling technique. Samples were gathered up to the necessary sample size was reached. The sampling area was selected purposefully based on the availability of HCV positive sample. The HCV positive samples were collected purposefully and HCV – negative sample was selected using systematic random sampling technique every K^{th}. The dried blood spot capacity were calculated using the formulas sensitivity, specificity, PPV and NPV, True Positive/True Positive + False Negative) x 100, True Negative/(True Negative + False Positive) x 100, True Positive/(True Positive +False Positive) x 100, True Negative/(True Negative + False Negative) x 100 respectively[28].

4.7 Measurement and Data Collection

4.7.1. Data collection procedure

Features of the socio-economic group and other study participants' variables were gathered by a standardized check list. The information gathering individuals were selected, oriented and trained to gather the information by check list. Venous blood DBS was created using a calibrated pipette on a different Whatman 903 protein-saver card from the EDTA tube. Whatman 903 protein-saver card is obtained from EPHI and manufacture of Eastern Business Forms 530 Old Sulphur Spring Rd, Greenville, SC29607, USA. 75 µl of venous blood were added into a Protein Saver 903 Card, until 12-mm circle fulfilled. These dried blood spot Cards were dried for at least

18 hours at room temperature before being sealed in zipped polyethylene bags containing Dry Desiccant and kept at -20°C until required.

4.7.2 Principles of Laboratory analysis

INNOTEST HCV AB IV is an enzyme immunoassay (EIA) for the detection of HCV antibodies in human serum or plasma. HCV synthetic peptides/recombinant proteins generated from distinct immunodominant areas are coated in the wells of poly styrene micro plate strips: the core (2 epitope clusters), NS3, NS4A, NS4B, and NS5A regions. These immunodominant areas' antigens come from a variety of HCV genotypes (1a, 1b, 2, 3a). If HCV-specific antibodies are present in a sample, they will attach to the antigens in the solid phase. Subsequently, an affinity – purified rabbit anti-human IgG (H chain specific) labeled with the enzyme horse radish peroxidase (HRP) is added. Up on a positive reaction this labeled antibody becomes bound to any solid –phase antigen/ antibody complex previously formed. Incubation with enzyme substrate produces a blue color in the test well, which turns yellow when the reaction is stopped with sulfuric acid. If the sample contain no HCV anti bodies, then the labeled antibody cannot be bound specifically and only a low background color develops [29].

WONDOFO rapid analysis- is immunochromatographic analysis and qualitative, membrane based immunoassay for the detection of antibodies to HCV in samples. Recombinant HCV antigen was membrane-coated in the cassette's test line region. Following examination, the sample interacts with colloidal gold, and an engineered HCV protein conjugate. A vibrant streak is created when the mixture moves laterally through the barrier by capillary action when it interacts with synthesized HCV protein. A vibrant streak absence denotes negative outcome and presence denotes positive outcome. As a procedural checked, a colored streaks will visible at checked streaks location[30].

4.7.3 Procedures and interpretation

Samples were gathered for detection of HCV infection from Ethiopian National Blood Bank Services. Anti-HCV–positive 159 positive and 159 negative anti HCV plasma samples were included in the investigation, along associated with DBS. Enzyme immunoassay techniques were used to confirm the existence of anti-HCV.

Rapid diagnosis test:-

Negative result: the collected sample whole blood/serum/plasma were used and add two drops of whole blood and two drops of dilution buffer or four drops of the serum/ plasma in the sample well and wait for 15minutes and interpret the result. Only one color band appears on the control (C) region. No apparent band on the test (T) region.

Positive result: In addition to a pink colored control (C) band, a distinct pink colored band will also appear in the test (T) region.

Invalid result: A total absence of color in both (C) and (T) regions and no colored band appears on the control (C) region is an indication of procedure error and/or the test reagent has deteriorated. Repeat with a new test kit [30].

DBS and PLASMA Preparation/ collection.

Venous blood DBS was produced using pipette in a different Whatman 903 protein-saver card from the EDTA tube. 75µl of venous blood were added into a Protein Saver 903 Card, which was then used to fill 12-mm circular card. These dried blood spot kit for18hrs at 25°C before being packed in separate zippered plastic bags with Dry Desiccant and until needed stored at -20°C. The leftover sample was used to prepare plasma by centrifugation at 3500rpm for 3minutes and separating the plasma within nunc tube by collecting all in to cry box and stored at-20 until used[31,32].

DBS Elution procedure.

Punch one spots from each dried blood spot of sample and pool to one eppendrof tube or nunc tube or ELISA plate. Add 200µl elution buffers. Between each sample, DBS punchers were cleaned by punching dry filter paper once, then four times with a filter saturated in 70% ethanol and to reduce cross contamination. At 1hr mixing eluted specimen done at 25°C with 500 rounds per minute, without shaking keeping overnight incubation and an additional hour of shaking at 500 rounds per minute[9,33].

4.8 Data Quality Assurance

Accuracy of the investigation ensured with applying quality assurance starting from the sample collection to result releasing.

Pre-analytical phase

Data accuracy was ensured by giving short training before data gathering, to data collectors and by following the prepared standard operating procedure (SOP) at various activities of the study. Sample labeling and identification were done with great care, and samples were stored in proper condition to ensure the quality. Clotted samples, wrong anticoagulant samples to transfer it to analytical phase.

Analytical phase

During performing a test the accuracy and expiration dates of equipment and reagents were first checked. The specimens' quality, collection, and serial number were also checked. The quality control was run by using 2 positive controls, 2 negative controls and 1 blank for each batch. Standard operating procedures (SOP) were followed when performing the analysis, with quality controls.

Post-analytical

The results of the laboratory testing were carefully documented in a well-prepared format. Using SPSS version 26 the finding was stored in secured area. Leftover samples were handled as per the laboratory regulation.

4.9 Data analysis and interpretation

Gained findings were entered and analyzed with SPSS software released 26. Descriptive data analysis like crosstab, correlate were used. The accurate relation was checked with 95% CI. The stored findings were saved in the form of text or tables. The lead investigator used an observation checklist for gathering data for the qualitative study.

4.10 Ethical considerations

The study was carried out after receiving conformation letter from the DRERC of Department of Medical Laboratory Sciences, College of Health Sciences Addis Ababa University. In addition, a recognition paper of collaboration was received from Department of Medical Laboratory Sciences to the analysis area. To maintain the confidentiality of all results, identification number were used.

4.11 Operational Definitions

DBS: are a dried blood spots which were collected from the venous blood on card.

HCV-antibody test: is the test used to find out if you are infected with the hepatitis c virus.

HCV: causes inflammation and liver disease

4.12 Dissemination of Results

The finding will be sent to AAUDML Science and accessible at the library for researchers and students. Additionally, this outcome will be shared for publishing in respected national and international publications and presentation at pertinent conferences.

5. Results

5.1 Population and sample characteristics

Of 318 participants screened, 131, 92,61, 24, and 10 were participated from Ethiopian National Blood Bank service, St. Peter Hospital, Yekatit 12 Hospital, Commercial Bank of Ethiopia Clinic and St. Paulo's Hospital Millennium Medical College, respectively. The participants' average years were 32 and 41% of them were females. The numbers of males were participated than females in this study and among age groups from 18-35years are participated than others (table1).

Table1. Socio demographic characteristics of study participant at Ethiopian National Blood Bank service, St. Peter Hospital, Yekatit 12 Hospital, Commercial Bank of Ethiopia Clinic and St. Paulo's Hospital Millennium Medical College, Addis Ababa Ethiopia, from June 2022 to March 2023

Characteristics		**Frequency**	**Percent**
Sex	F	129	40.6
	M	189	59.4
Age	18-25	107	33.6
	26-35	102	32.1
	36-45	68	21.4
	46-55	33	10.4
	>56	8	2.5
	Total	318	100.0

Of 318 HCV tested 78negative and 53positive, 50negative and 42positive, 31negative 30positive, 24positive and 10positive sample were collected from Ethiopian National Blood Bank service, St. Peter Hospital, Yekatit 12 Hospital, Commercial Bank of Ethiopia Clinic and St. Paulo's Hospital Millennium Medical College, respectively. 159(50%) RDT, 159(50%) ELISA and 156(49.1%) RDT DBS sample among 158(49.7%) ELISA DBS samples were positive the remaining were negative 159(50%), 159 (50%), 162(50. 9%) and 158(49.7%) respectively (table2).

Table 2. Evaluation of DBS, RDT DBS and ELISA at Ethiopian National Blood Bank service, St. Peter Hospital, Yekatit 12 Hospital, Commercial Bank of Ethiopia Clinic and St. Paulo's Hospital Millennium Medical College, Addis Ababa Ethiopia, from June 2022 to March 2023

Types of tests	**Result**		Total
	Positive n(%)	**Negative n(%)**	
RDT result	159 (50%)	159(50%)	318(100%)
RDT DBS sample result	156(49.1%)	162(50. 9%)	318(100%)
ELISA Plasma samples result	159 (50%)	159 (50%)	318(100%)
ELISA DBS samples result	158(49.7%)	160 (50.3%)	318(100%)

5.2 Performance of DBS among RDT, RDT DBS and ELISA

Analyzing DBS sensitivity, specificity, PPV,NPV and using plasma tests as the reference method allowed for an evaluation of the diagnostic capacity of the DBS employed to screen for HCV(Table3, Table4 &Table5) among RDT were 100%, 99.4%, 99.4%, 100%, RDT DBS were 98.7%, 99.4%, 98.7%, 99.4% and ELISA were 100%, 99.4%, 99.4%, 100% respectively (table4, table5). Based on the WHO recommendation, pooled sensitivity for anti-HCV detection antibodies Specificity and sensitivity were 99% and 98% [21]. Therefore, this study shows that DBS was an alternate sample for anti-HCV detection.

Table 3.ELISA DBS samples result and ELISA Plasma samples result at Ethiopian National Blood Bank service, St. Peter Hospital, Yekatit 12 Hospital, Commercial Bank of Ethiopia Clinic and St. Paulo's Hospital Millennium Medical College, Addis Ababa Ethiopia, from June 2022 to March 2023

		ELISA Plasma samples result		total
		Negative n(%)	Positive n(%)	
ELISA DBS samples result	Negative	159(99.4%)	1(0.6%)	160(100%)
	Positive	0(0.0%)	158(100%)	158(100%)
	Total	159(50%)	159(50%)	318(100%)

Table 4. ELISA DBS samples result and RDT DBS sample result at Ethiopian National Blood Bank service, St. Peter Hospital, Yekatit 12 Hospital, Commercial Bank of Ethiopia Clinic and St. Paulo's Hospital Millennium Medical College, Addis Ababa Ethiopia, from June 2022 to March 2023

		RDT DBS sample result		Total
		Negative n(%)	Positive n(%)	
ELISA DBS samples result	Negative	160 (99.4%)	0(0.o%)	160(100%)
	Positive	2(1.3%)	156(98.7%)	158(100%)
	Total	162(50.9%)	156(49.1%)	318(100%)

5.4 Association of DBS among RDT, RDT DBS and ELISA

Our study indicates there was strong association between DBS and RDT DBS, ELISA and RDT as strong association was range from 0.7 to 1 and there association result was 0. 963, 0. 969 and 0. 969 respectively. P value < 0.05 the sig result was 0.00 this indicates significant association.

6. Discussion

This finding was done to assess the DBS versus plasma sample with HCV test results done by using ELISA and RDT diagnostic methods at Ethiopian National Blood Bank Service, Addis Ababa, Ethiopia. The findings was positivity and negativity rate of RDT, ELISA, DBS and RDTDBS were 159(50%) and 159(50%), 159(50%) and 159(50%), 158(49.7%) and 160(50.3%), and 156(49.1%), 162(50.9%). Sensitivity, specificity, PPV, NPV, DBS sample among RDT, RDT DBS and ELISA were 100%, 99.4%, 99.4%,100%,100%, 99.4%, 99.4%, 100%, 98.7%, 99.4%, 98.7%, 99.4% respectively. Correlation between DBS among RDTDBS, ELISA and RDT were 0.963,0.969 and 0.969 and indicates significantly associated with DBS sample.

In this study, 18-35 years old of age were at risk of HCV. This is consistent with one study show on Cross-Sectional Study[34]. This might be The majority of these new infections affect young adults who use heroin injectable; many of them switched from taking prescription medications, because it's frequently cheaper and easier to obtain[34].

According to this study, men had a greater risk to get HCV positive than females similar with investigation done on new face hepatitis C virus [35]. This may be because men have a greater possibility than women to be exposed to occupational diseases related to agricultural work[34].

In present study, of 318 individual participated in the study were equal number of (half of total) HCV positive and HCV negative were detected using ELISA and RDT with the same sample for detection of HCV. Study conducted in 2021 ELISA was taken as confirmatory serological assay for detection of HCV and the results of RDT were compared and has the same result [22]. This might be duet the same method were used.

In our study 318 individual were tested for HCV, using ELISA, RDT and DBS. Of these, the same number of samples was found to be positive by ELISA and RDT, except one result opposite for DBS with the same sample. This study consistence with study done on prospective assessment of hepatitis C virus antibody detection in whole blood drown on DBS with the INNOTEST® HCV Ab IV enzyme immunoassay due to the same taste method were used[9].

High sensitivity, specificity, PPV and NPV of DBS were reported from this study and this study similar with study done on; future assessment of hepatitis C virus antibody detection in whole blood collected on DBS with the INNOTEST® HCV Ab IV enzyme immunoassay[9,36]. This was due to similar test method were used. This suggests that the analyzer works effectively in dried blood spot samples that contain antibodies to HCV at naturally occurring levels. Despite the fact that shakers and rotors are frequently used in laboratories, the test calls for the ability to shake samples for two hours at a constant rate. Laboratories that don't already have access to these supplies could have to make a one-time investment to get what they require. DBS punchers are available in stationery stores, or normal whole punchers with a 6 mm diameter can be used in their place.

This study indicates the test result correlation agreement between of DBS among ELSA & DBS-RDT was 0. 969 & 0. 963 respectively and this were relatively the same to Study done in Ukraine [36].

7. Limitation

The limitations of this finding include; The INNOTEST HCV Ab IV assay procedure was designed to detect antibodies to HCV in human plasma and serum. Insufficient data are available to interpret tests performed on other body fluids. Therefore, testing of such specimens is not recommended.

A negative result with the INNOTEST HCV Ab IV does not preclude the possibility of exposure to, or infection by, the hepatitis C virus. Levels of antibodies to HCV may be undetectable in early infection. The presence of antibodies to HCV does not constitute a diagnosis of hepatitis C but may be indicative of recent and/or past infection by hepatitis C virus.

8. Conclusion and recommendation

8.1. Conclusion

This finding indicates, entire blood obtained with a dried blood spot using for both monitoring and diagnosing HCV infection. Since the INNOTEST® HCV Ab IV assay performed effectively in DBS and using dried blood spot increase health care monitoring.

8.2. Recommendation

Other researcher can perform DBS evaluation using this assay including all characteristics of the individual and using DBS as an alternative sample in resource limited resource country.

8. References

1. The World Health Organisation. Guidelines on hepatitis B and C testing., Guidelines on hepatitis B and C testing. 2017(66). 1–170 . http://apps.who.int/iris/bitstream/10665/254621/1/9789241549981-eng.pdf?ua=1%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/28742301%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/28742301

2. The Netherlands Is on Track to Meet the World Health Organization Hepatitis C Elimination Targets by 2030_Enhanced Reader.pdf. J. Clin. Med. **2021**;doi.org/10.3390/jcm10194562

3. Graham CS, Swan T. A path to eradication of hepatitis C in low- and middle-income countries. Antiviral Res 2015;119:89–96. dx.doi.org/10.1016/j.antiviral.2015.01.004

4. Easterbrook PJ, Roberts T, Sands A, Peeling R. Diagnosis of viral hepatitis. 2020;12(3).

5. Belyhun Y, Maier M, Mulu A, Diro E, Liebert UG. Hepatitis viruses in Ethiopia: A systematic review and meta-analysis. BMC Infect Dis 2016;16(1):1–14. dx.doi.org/10.1186/s12879-016-2090-1

6. Thursz M, Lacombe K. Breaking down barriers to care in hepatitis C virus infection. J Infect Dis 2016;213(7):1055–6.

7. Id JAC, Carlos J, Higuita- LF. Prevalence of hepatitis B / C viruses and associated factors in key groups attending a health services institution in Colombia 2020;1–14.

8. WHO. Executive summary - Global hepatitis report. World Heal Organ 2017; apps.who.int/iris/rest/bitstreams/1082595/retrieve

9. Vetter BN, Reipold EI, Ongarello S, Fajardo E, Tyshkovskiy A, Ben I, et al. Prospective evaluation of hepatitis C virus antibody detection in whole blood

collected on dried blood spots with the INNOTEST® HCV Ab IV enzyme immunoassay. J Clin Virol 2021;137:104783.

10. Dokubo EK, Evans J, Winkelman V, Cyrus S, Tobler LH, Asher A, et al. Comparison of Hepatitis C Virus RNA and antibody detection in dried blood spots and plasma specimens. J Clin Virol 2014;59(4):223–7.

11. Ma J, He L, Chen K, Wang Y, Ren Y, Xing W, et al. Biosafety and Health Comparative study on anti-HCV testing using plasma , dried plasma spots (DPS), and dried blood spots (DBS). Biosaf Heal 2020;(xxxx).

12. Muzembo BA, Mbendi NC, Nakayama SF. Systematic review with meta-analysis: performance of dried blood spots for hepatitis C antibodies detection. Public Health 2017;153:128–36. doi.org/10.1016/j.puhe.2017.08.008

13. Vázquez-morón S, Jiménez BA, Jiménez-sousa MA, Bellón JM, Ryan P, Resino S. Evaluation of the diagnostic accuracy of laboratory-based screening for hepatitis C in dried blood spot samples : A systematic review and meta-analysis. 2019;(October 2018):1–12.

14. Lange B, Cohn J, Roberts T, Camp J, Chauffour J, Gummadi N, et al. Diagnostic accuracy of serological diagnosis of hepatitis C and B using dried blood spot samples (DBS): Two systematic reviews and meta-analyses. BMC Infect Dis. 2017;17(Suppl 1).

15. Crimmins EM, Zhang YS, Kim JK, Frochen S, Kang H, Shim H, et al. Dried blood spots: Effects of less than optimal collection, shipping time, heat, and humidity. Am J Hum Biol. 2020;32(5):1–7.

16. A Lange B, Roberts T, Cohn J, Greenman J, Camp J, Ishizaki A, et al. Diagnostic accuracy of detection and quantification of HBV-DNA and HCV-RNA using dried blood spot (DBS) samples - a systematic review and meta-analysis. BMC Infect Dis. 2017;17.

17. Fondjo CLK, Ngoupo PAT, Ngono L, Plantier JC, Njouom R. Performance evaluation of three rapid screening assays for detection of antibodies to hepatitis C virus in Cameroon. BMC Res Notes. 2018;11(1):1–4.

18. Poiteau L, Soulier A, Rosa I, Roudot-Thoraval F, Hézode C, Pawlotsky JM, et al. Performance of rapid diagnostic tests for the detection of antibodies to hepatitis C virus in whole blood collected on dried blood spots. J Viral Hepat. 2016;23(5):399–401.

19. Kenmoe S, Tagnouokam PAN, Nde CK, Mella-Tamko GF, Njouom R. Using dried blood spot for the detection of HBsAg and anti-HCV antibodies in Cameroon. BMC Res Notes. 2018;11(1):1–4. doi.org/10.1186/s13104-018-3931-3

20. Platt L, Easterbrook P, Gower E, Mcdonald B, Sabin K, Mcgowan C, et al. Prevalence and burden of HCV co-infection in people living with HIV : a global systematic review and meta-analysis. Lancet Infect Dis 2016;3099(15):1–11. dx.doi.org/10.1016/S1473-3099(15)00485-5

21. Holzmayer V, ATaylor R, Kuhns MC, Gawel SH, Ndembi N, Mbanya D, et al. Evaluation of hepatitis C virus antibody assay using dried blood spot samples., Scientific Reports. Nature Publishing Group UK; 2022(12). doi.org/10.1038/s41598-022-07821-0

22. Ostler MW, Porter JH, Buxton OM. Dried blood spot collection of health biomarkers to maximize participation in population studies. J Vis Exp. 2014;(83):1–9.

23. Vázquez-Morón S, Ryan P, Ardizone-Jiménez B, Martín D, Troya J, Cuevas G, et al. Evaluation of dried blood spot samples for screening of hepatitis C and human immunodeficiency virus in a real-world setting.Scientific Reports. 2018;8

24. Malone L, Cm MBA, Grigorenko E, Stalons D. Comparative study on anti-HCV testing using plasma, dried plasma spots (DPS), and dried blood spots (DBS) Id Week 2015. 2017;2.

25. Lee CE, Sri Ponnampalavanar S, Syed Omar SF, Mahadeva S, Ong LY, Kamarulzaman A. Evaluation of the dried blood spot (DBS) collection method as a tool for detection of HIV Ag/Ab, HBsAG, anti-HBs and anti-HCV in a malaysian tertiary referral hospital. Ann Acad Med Singapore. 2011;40(10):448–53.

26. Kania D, Bekalé AM, Nagot N, Mondain AM, Ottomani L, Meda N, et al. Combining rapid diagnostic tests and dried blood spot assays for point-of-care testing of human immunodeficiency virus, hepatitis B and hepatitis C infections in burkina faso, west africa. Clin Microbiol Infect. 2013;19(12):12–8.

27. CLSI. CLSI M100-ED29: 2021 Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition., Clsi. 2020(40). 50–51 .

28. Wayne PA. Validation, Verification, and Quality Assurance of Automated Hematology Analyzers, 2nd Edition. Clin Lab Stand Inst H26-A2. 2010;30(14). clsi.org/standards/products/hematology/documents/h26/

29. Pq WHO, Report P, Hcv I, Iv A, Nv FE, Innotest T, et al. WHO Prequalification of In Vitro Diagnostics PUBLIC REPORT Product : INNOTEST HCV Ab IV WHO reference number : PQDx 0201-073-00. 2018;1–20.

30. Development A, Hepatitis V, Kamili S. Hepatitis C Antibody / Hepatitis C Serum Confirmatory Test Laboratory Procedure Manual. 2017.

31. Winter O, Beijing G. COLLECTION OF A DRIED BLOOD SPOT (DBS) OLYMPIC WINTER GAMES BEIJING 2022. 2022;(January).

32. Receiving I. Guide for Receiving and Storing Dried Blood Spots (DBS) for Molecular Testing at Reference Laboratories Guide for Receiving and Storing Dried Blood Spots (DBS) for Molecular Testing at Reference Laboratories Storage Steps :1–4.

33. WHO guidelines on hepatitis B and C testing. Geneva: World Health

Organization; 2017. apps.who.int/iris.

34. Araujo AC, Astrakhantseva I V., Fields HA, Kamili S. Distinguishing acute from chronic hepatitis C virus (HCV) infection based on antibody reactivities to specific HCV structural and nonstructural proteins. J Clin Microbiol. 2011;49(1):54–7.

35. Study C, Alharazi T, Alzubiery TK, Alcantara JC, Qanash H. Syphilis and Malaria) in Blood Donors : A Large-Scale. 2022;

36. Sun C, Iwamoto M, Calzia A, Sreng B, Yann S, Pin S, et al. Demonstration of the diagnostic agreement of capillary and venous blood samples, using hepatitis-C virus SD Bioline© rapid test: A clinic-based study. J Clin Virol. 2019;111:39 doi.org/10.1016/j.jcv.2018.12.008

Annex: I English Version Check list

Participant Code/MRN: _____

Check list for Evaluation of the DBS sample for Detection of HCV– Antibody at Ethiopian National Blood Bank service and International Clinical Laboratory Addis Ababa, 2022

The following questions are targeted to differentiate question

Please circle the best proper choice of answer code.

Sample label number(code)_____

No.	Questions	Coding Classification	Remark
101	Gender	Male = 1 ,Female = 2	
102	Age in years	_____years	
103	Address	Region_____zone/subcity_____ woreda_____kebele_____	
104	HCV status	Positive=1 Negative =2	
105	DBS done?	1= Yes , 2= No	
106	Plasma separated?	1= Yes , 2= No	
107	ELISA done?	1= Yes , 2= No	
108	ELISA result	Positive=1 Negative =2	
109	Does discordant present?	1= Yes , 2= No if discordant is present do PCR otherwise skip it	
110	PCR done?	1= Yes , 2= No	
111	PCR result	Positive=1 Negative =2	

Annex: IIstandard operative procedure for INNOTEST HCV Ab IV

Purpose: This procedure provides instructions for INNOTEST HCV Ab IV

Abbreviations: HCV: Hepatitis c virus

N/A: Not applicable

Definition: The INNOTEST HCV Ab IV is an enzyme immunoassay for the detection of antibodies to human Hepatitis C virus (HCV) in human serum or plasma.

Principle: HCV synthetic peptides/recombinant proteins generated from distinct immunodominant areas are coated in the wells of poly styrene micro plate strips: the core (2 epitope clusters), NS3, NS4A, NS4B, and NS5A regions. These immunodominant areas' antigens come from a variety of HCV genotypes (1a, 1b, 2, 3a). In such a well, the test sample is incubated. If HCV-specific antibodies are present in a sample, they will attach to the antigens in the solid phase. Subsequently, an affinity – purified rabbit anti-human IgG (H chain specific) labeled with the enzyme horse radish peroxidase (HRP) is added. Up on a positive reaction this labeled antibody becomes bound to any solid –phase antigen/ antibody complex previously formed. Incubation with enzyme substrate produces a blue color in the test well, which turns yellow when the reaction is stopped with sulfuric acid. If the sample contain no HCV anti bodies, then the labeled antibody cannot be bound specifically and only a low background color develops.

Materials and Reagents

Description, preparation for use and recommended storage conditions

- It kept at 2 to 8^0C, all test reagents, including the coated tst wells, are stable until the expiry date given on the pack.

Each pack contains:

1. 1. Vial containing 1.5 mL (192T) or 3 mL (480T) of negative control (human serum containing 0.01% menthylisothiazolone (MIT) and <0.1% chloracetamide (CAA) as preservative).

2. 1 vail containing 1.5 mL (192T) or 3 ML (480T) of positive control (phosphate buffer containing antibodies to HCV, protein stabilizers, and proclin 300 as preservative).

Note: precipitation may be seen in the positive control. This precipitation has no impact on test performance and results.

3. 2 sachets (192T) or 5 sachets (480T) containing a strip-holder with 12x8 HCV antigen-coated test wells (plate).

A silicagel bag is added as dessicant.

After opening the aluminium foil bag containing the strips, any unusedtest wells will be stable for 8 weeks if stored at 2 to 8^0C in the closed plastic minigrip bag with the silicagel.

4. 1 vial containing 60 mL (192T) or 150 ML (480T) sample diluent (phosphate buffer containing sodium chloride, Trito, proten stabilizers and proclin 300 as preservative purple colored buffer solution).
5. 1 vial containing 150 mL (192T) or 200 mL (480T) of concentrated wash solution (phosphate buffer containing 0.01% MIT and <0.1% CAA as preservative), to be diluted 1:25 with distilled or deionized water before use.

$No\ of\ tests$	$wash\ solution\ (ML)$	$H_2O\ (mL)$
$1x69$	$40 + 960$	
$5x96$	$200 + 4800$	

NOTE: Salt crystals may be formed in the concentrated wash solution after storage at 2 to 8^0C. These crystals should be completely redissolved, by warming at 37^0C, before dilution.

Diluted wash solution is stable for 4 weeks if stored at 2 to 8^0C.

6. 1 vial containing 60 mL (192T) or 150mL (480T) conjugate diluent (phosphate buffer containing protein and enzyme stabilizers and 0.05% Proclin 300 as preservative green colored buffer solution)>
7. 1 vial containing 0.60 mL (192T) or 1.5 mL (480T) of concentrated conjugate 100x (rabbit anti-human IgG (H chain) labeled with horseradish peroxidase), to be diluted 1:100 with conjugate diluent before use.

Number of tests	8	16	32	64	96	2x96	5x96

Conjugate (mL)	0.020	0.040	0.080	0.160	0.240	0.480	1.200
Conjugate diluent (mL)	2	4	8	16	24	48	120

Conjugate working soilution is stable for 8 hours if stored at room temperature.

8. 1 vial containing 60 mL (192T) or 150 mL (480T) of substrate buffer (phosphate citrate buffer containing 0.006% hydrogen peroxide).
9. 1 vial containing 1 mL(192T) or 1.5mL (480T) of concentrated substrate TMB 100X(teramethylbenzidine (TMB) dissolved in dimethylsulfoxide (DMSO)), to be diluted 1:100 before use.

Number of tests	8	16	32	64	96	2x96	5x96
substrate (mL)	0.020	0.040	0.080	0.160	0.240	0.480	1.200
Substrate buffer (mL)	2	4	8	16	24	48	120

Note: concentrated substrate TMB should be melted completely (melting point 18^0C).

Substrate solution is stable at room temperature (18 to 30^0C) for 1 hour if kept in the dark.

10.1 vial containing 30 mL (192T) or 45 mL (480T) stop solution (0.9 N sulfuric acid).

11.8 (192T) or 15 (480T) adhesive plate sealers.

12.1 (192T) or 2 (480T) plastic minigrip bag (s) for storage of unused strips.

- Distilled or deionized water.
- Precision pipettes with disposable tip to deliver in the ranges of 20 to 200 pL, and 200 to 1000 pL, respectively.
- Optionally a multichannel pipette to deliver 200 pL can be used together with disposable V-shaped troughs for addition of conjugate, substrate solution and stop solution.
- Microplate shaker.
- Incubator at 37°C.
- Microplate washer (alternatively, washing can be performed manually, e.g. by using a repeating syringe delivering 400 pL volumes and an aspirating device).

- Absorbent tissues.
- Photometric reading: microplate reader, equipped with a 450 nm filter and optional 620 nm filter.

Specimen:- Serum or plasma, containing heparin, citrate and EDTA as anticoagulant, can be used. Please note there will be no SAM (Sample Addition Monitoring) color change of the sample diluent when using EDTA-plasma specimens.

- Specimens should be free of microbial contamination when tested.
- Additives may give erroneous results.
- Insoluble material should be removed from all samples by centrifugation before testing.
- Before storage, serum or plasma should be separated from blood clot or blood cells by centrifugation.
- Store the samples at 2 to 8°C. For storage longer than one week, freeze in aliquots at-20°C.
- Do not use any heat-treated specimens.

Environmental and safety controls

Please refer to the Safety Data Sheet (SDS) and product labeling for information on potentially hazardous components. The most recent SDS version is available on the website www.fuiirebio-europe.com.

Warning Contains mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one

H317 P261 P280 P362+P364 P333+P313 P302+P352

Contains dim ethyl sulfoxide

H315 H319 H335 P280 P261 P305+P351+P338 P362+P364 P312 P302+P352

Hazard statement

H315 Causes skin irritation.

H317 May cause an allergic skin reaction.

H319 Causes serious eye irritation.

H335 May cause respiratory irritation.

Precautionary statements

P261 Avoid breathing mist/vapours/spray.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P302+ P352 IF ON SKIN: Wash with plenty of water/...

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P312 Call a POISON CENTER/doctor/.../ if you feel unwell.

P333+P313 If skin irritation or rash occurs: Get medical advice/attention.

P362+P364 Take off contaminated clothing and wash it before reuse

- Only adequately trained personnel should be permitted to perform the test procedure.
- Specimens and negative control should always be handled as potentially infectious.
- The positive control has been found to be negative for anti-HIV-1/HIV-2 and HBsAg. The negative control has been found to be negative for anti-HIV-1/HIV-2, anti-HCV and HBsAg.

No test method can offer complete insurance that blood products will not transmit infectious agents. Therefore, all blood components and biological materials should be considered as being potentially infectious and should be handled as such.

All blood components and biological materials should be disposed of in accordance with established safety procedures.

- Autoclave for at least 15 minutes at 121°C.
- Incinerate disposable material.

- Mix liquid waste with sodium hypochorite so that the final concentration is ± 1% sodium hypochlorite. Allow to stand overnight before disposal.
- Caution: Neutralize liquid waste that contains acid before adding sodium hypochlorite.

- Avoid contact and inhalation of TMB substrate solution. This solution is irritating to the skin, eyes and the respiratory system, and it may also cause sensitisation by inhalation and skin contact. TMB substrate solution, containing DMSO, provokes very rapid absorption by skin.
- Negative control and wash solution contain MIT/CAA as preservative. This solution may cause sensitisation by skin contact. Wear gloves and protective goggles.
- The kit contains 0.9N sulfuric acid as stop solution.
- Use of personal protective equipment is necessary: gloves and safety spectacles when manipulating dangerous or infectious agents.
- Waste should be handled according to the institution's waste disposal guidelines. All federal, state, and local environmental regulations should also be observed.

Procedure

Please read 'Remarks and precautions' before performing the test.

All test materials must be brought to room temperature (18 to 30°C) approximately 30 minutes before use and returned to the refrigerator (2 to 8°C) immediately after use. To avoid water condensation into the wells, the aluminum foil bag must be kept closed until the device is stabilized at room temperature.

Before starting the assay, adjust the temperature of the incubator to 37 ± 1°C.

1. Take the strip-holder with the required number of strips, ensuring that for one strip, one SAM control well, one negative and one positive control should be included; for more strips, at least one SAM control well, two negative and two positive controls should be included in each strip holder. During the test run, strips stay in the strip- holder and can be marked on one edge.

2. Add 200 µL of sample diluent to each test well including the SAM control well.

3. Add 20 µL of specimen or control to each appropriate test well, except to the SAM control well. A color change from purple to dark blue indicates that the specimen or control has been

added to the micro well.

The SAM color change can also be read photo metrically at a wavelength of 620 nm:

- Blank the reader on the SAM control well according to the instrument manufacturer's instructions.
- Each control or specimen should exhibit a value of greater than or equal to 0.100.

Make sure specimens and controls are adequately mixed with the sample diluent by pipetting up and down 5 times or by using a plate shaker at 1000 rpm for 1 minute.

4. **Cover** the strips with an adhesive sealer. **Incubate** for 60 ± 3 minutes at 37 ± 1°C. Note: Prepare conjugate working solution during incubation, see "Reagents".

5. **Wash** each well 6 times (see “Directions for washing”).

6. Add **200 µL** prepared **conjugate working solution** to each well including the SAM control well.

A photometric read at a wavelength of 450 nm to document conjugate addition can be performed after addition of conjugate working solution to the microwell strips:

- Do NOT blank the reader on the SAM control well.
- Each control or specimen should exhibit a value of greater than or equal to 0.950.

7. Cover the strips with a new adhesive sealer. Incubate for 60 + 3 minutes at 37 ±1°C.

Note: Prepare substrate solution during incubation, see “Reagents".

8. Wash each well 6 times (see “Directions for washing”).

9. Add 200 pL prepared substrate solution to each well.

10. Incubate for 30 ± 1 minutes at room temperature in the dark.

11. To stop the reaction, add 50 JJL stop solution to each well in the same sequence and at the same time intervals as the substrate solution. Tap the strip holder carefully to ensure thorough mixing.

12. **Read** the absorbance of the solution in the wells within 15 minutes after step 11 at 450 nm with a microplate reader. **Do NOT blank the reader on the SAM control well.**

Directions for washing

Pre-rinse the washer with diluted wash solution.

Perform **manual wash** as follows:

- Aspirate completely the liquid from all wells by lowering an aspiration tip gently to the bottom of each well.
- Take care not to scratch the inside of the well surface.
- After each aspiration invert the plate and tap it dry on absorbent tissue.
- Fill the wells with 400 (JL wash solution.
- Leave to soak for a minimum of 30 seconds, then aspirate the liquid
- Perform the step 6 times.

In the absence of recommended washer or protocol, carry out automatic washing as follows: Perform 6 wash cycles ensuring that:

- The fill volume is 400 pL/well.
- The dispensing height is set to completely fill the well.
- The time taken to complete one aspiration/wash/soak cycle is approximately 30 seconds.

Incomplete washing will adversely affect the test outcome. Contamination of wash solution and washer can cause extensive problems. In case problems occur, desinfect the wash bottles and washer overnight with a 4% formaldehyde solution.

Result Interpretation

Abbreviations:

P = mean of the absorbance of positive controls

S = mean absorbance of the test sample

Validation

Check the validity of individual negative and positive controls (absorbances at 450 nm).

- Each of the negative controls should be lower than 0.100.
- Each of the positive controls should be higher than 0.800.
- Calculate P eliminating controls under 0.800.

If more than half the numbers of controls have to be eliminated, the test run should be repeated after careful investigation into the source of errors.

Test result

Calculate the cut-off value as: (P/2.75).

A sample is NON-REACTIVE if S < (P/2.75).

A sample is REACTIVE if S ≥ (P/2.75).

IMPORTANT REMARK:

It is advised not to make a correction for a blank. This is because samples which are borderline positive before correction can become borderline negative afterwards. In this case, all OD values are lowered with the OD-value of the blank; the value of the cut-off is only lowered with the blank value divided by 2.75.

A sample reactive upon initial testing must be retested in duplicate before results interpretation.

A repeatedly reactive sample must be confirmed with an additional confirmatory test.

Limitations

The INNOTEST HCV Ab IV assay procedure was designed to detect antibodies to HCV in human plasma and serum. Insufficient data are available to interpret tests performed on other body fluids. Therefore, testing of such specimens is not recommended.

A negative result with the INNOTEST HCV Ab IV does not preclude the possibility of

exposure to, or infection by, the hepatitis C virus. Levels of antibodies to HCV may be undetectable in early infection. The presence of antibodies to HCV does not constitute a diagnosis of hepatitis C but may be indicative of recent and/or past infection by hepatitis C virus.

References

Pq WHO, Report P, Hcv I, Iv A, Nv FE, Innotest T, et al. WHO Prequalification of In Vitro Diagnostics PUBLIC REPORT Product : INNOTEST HCV Ab IV WHO reference number : PQDx 0201-073-00. 2018.

Annex III standard operative procedure for dried blood spot

Purpose: This procedure provides instructions for collecting, packaging, storing and shipping samples collected as dried blood spots (DBS).

Definition: is a form of bio sampling where blood samples are blotted and dried on filter paper and easily be shipped to a laboratory and analyzed using various methods such as DNA amplification.

Abbreviations: DBS:dried blood spots

N/A: Not applicable

Principle: Dried blood spot or dried blood sampling (DBS) is an innovative sampling technique where small blood samples are blotted on an absorbent paper and allowed to dry for analysis.

Materials and Reagents

1. Materials for all methods of specimen collection

- ✓ Whatman Protein Saver Card #903 (Whatman #10534612; Fisher Scientific #NC9307519)
- ✓ Alcohol swab

Note: These guidelines refer specifically to DBS created from Whatman 903 paper. Other types of DBS paper are available (e.g. FTA-DMPK-A, FTA-DMPK-B, FTA-DMPK-C) and may be designated in a given protocol. Specific instructions with respect to blood volume, storage conditions and extraction methods should be provided in the protocol or by the manufacturer. Labeling and packaging requirements outlined in this document should be followed regardless of the paper used. Customized card configurations may be specified within a protocol. Ensure that the appropriate cards are available before collecting any specimens.

- ✓ Gas-impermeable storage bag
- ✓ Desiccant pack
- ✓ Humidity indicator Cards Whatman card drying rack Gloves, preferably powder-free
- ✓ Waterproof marker
- ✓ Glassine Envelopes, 3 1/4 inches x 4 7/8 inches, 100-pack ; optional

2. Materials specifically needed for heel stick method

- ✓ Unistick 2 device
- ✓ Dry sterile gauze pad

3. Materials specifically needed for finger stick method

- ✓ Lancet/needle

4. Materials specifically needed for venous blood method using Vacutainer® Tubes

- ✓ Vacutainer Evacuated Blood Collection Tubes
- ✓ Tourniquet
- ✓ Bandage/plaster
- ✓ Vacutainer Needle
- ✓ Vacutainer Needle holder

Specimen

A. Collection of dried blood spots from heel stick

The Unistick 2 device has a penetration depth of 1.8 mm and a convex tip for accurate positioning to eliminate the risk of accidental needle stick injury or cross-infection and reduce the risk of osteomyelitis of the heel.

- ✓ Clearly label the Whatman Protein Saver 903 Card/spots with Patient ID and Date, Protocol, Visit ID using a marker or LDMS generated label(s).

Note: DBS cards collected for use in ACTG and IMPAACT must include LDMS labels.

- ✓ Prepare the Unistick 2 device by depressing the pink plunger until it clicks. Twist the plunger until it breaks off and remove it from the device. The Unistick 2 device is now ready to trigger.
- ✓ Preferably, the baby should be in a supine position (lying down face up) with the knee at the edge of the table. This position allows for the foot to hang lower than the torso, improving blood flow. Alternatively, the baby may be held in the mother's arms.
- ✓ Clean the incision area of the heel with an alcohol pad and allow the heel to air dry. Do not touch the incision site or allow the heel to come into contact with any non-sterile item or surface.

- ✓ Wrap your index finger around the base of the heel and your thumb around the ankle.
- ✓ Position the device properly against the outside of the heel or big toe
    - Draw an imaginary line from midpoint of the big toe to the heel and one from between the 4th and 5th toe to the heel.
    - Black areas indicates safe areas for puncture site
    - Do not puncture the back of the heel or Achilles tendon or the medial aspect of the big toe. Solid red areas indicate areas that are not safe for puncture
- ✓ Trigger the Unistick 2 heel-stick device and wipe away the first drop of blood using a dry sterile gauze pad.
- ✓ Hold the Whatman Protein Saver 903 Card without touching the filter paper
- ✓ Allow the blood from the heel to flow for collection onto the dried blood spot card by gently touching the filter paper card to the blood drop. Allow the card to absorb the blood until the circle is full. You should be able to obtain 5 spots of blood on a card. (You may need to squeeze the heel to obtain more blood. However, do not milk the heel or interstitial fluid will be mixed in with the blood.) Do not touch the DBS circle once blood is applied.
- ✓ Gently press a sterile pad to the incision site until bleeding stops.
- ✓ Monitor the baby's heel for late bleeding and inflammation. Place a sterile pad over the wound to prevent formation of a hematoma.
- ✓ Note: Bandaging the baby's foot is controversial because of skin sensitivity. The incision should be monitored by the primary care nurse for bleeding andinflammation. A bandage is not necessary as long as the bleeding has stopped before the child leaves the clinic.

B. Collection of dried blood spots from finger stick

- ✓ Clearly label the Whatman Protein Saver 903 Card with Patient ID and Date, Protocol, Visit ID using a marker or LDMS generated label(s).

Note: DBS cards collected for use in ACTG and IMPAACT must include LDMS labels.

- ✓ Disinfect selected site on the finger and prick using a lancet/needle
- ✓ Hold the Whatman Protein Saver 903 Card without touching the filter paper

- ✓ Uniformly saturate the entire circle by quickly and gently touching the drop of blood to the Whatman Protein Saver 903 Card. Do not press the puncture site to the filter paper or touch the Whatman Protein Saver 903 Card at any stage of collection. Do not touch the DBS circle once blood is applied.
- ✓ After collecting 5 dried blood spots, clean the site and leave it un-bandaged and dry spacimen

C. Collection of venous blood using EDTA tube

- ✓ Vacutainer Evacuated Blood Collection Tubes are designed to be filled with a predetermined volume of blood by vacuum. The rubber stoppers are color-coded according to the additive that the tube contains. Collect 1 EDTA (purple top) tube at each required time point. The total volume required per card will be 1 x 0.250 or 5 x .050 mL per spot (for 50 µL spots) or 1 x 0.400 mL or 5 x 0.080 mL per spot (for 80 µL spots). The total blood volume needed is dependent on the number of spots to be created for each sample.
- ✓ Label the tubes with the patient ID number, the date of collection, and the study and visit identifier using a marker or LDMS generated label(s).
- ✓ Fill the blood collection tube to the recommended volume so the anticoagulant is at the proper dilution.
- ✓ Gently invert the tube (8 to 10 times) to mix the blood thoroughly. After the blood is completely mixed, remove the cap, and apply 50 to80 µL of the whole blood to a single spot on the Whatman Protein Saver 903 Card, do not touch the card with the pipette tip. Slowly expel blood from the tip and touch the drop to the paper, allowing the blood to absorb. Care must be taken when applying larger volumes of blood to ensure the spots do not run outside the circle. Repeat four times to fill all five spots on the card. This transfer of blood should be performed with a pipette and a disposable tip. A single tip may be used to load the card; cover and mix blood by inversion in between applications if creating more than one DBS card. Do not touch the DBS circle once blood is applied..

D. Specimen Drying

- ✓ Allow the blood spot to air dry without the card flap covering the spots in a clean, dry place (i.e. biosafety cabinet,) that is protected from rodents, insects and direct sunlight

for at least 4 hours (drying overnight may be necessary in areas with higher humidity; less drying time may be warranted for some analytes, e.g. malaria parasites). Do not heat, stack or allow DBS to touch other surfaces during the drying process

- ✓ Tuck in the flap of the Whatman Protein Saver 903 Card as indicated on the card. If the DBS card does not have a protector flap, it is recommended to put the DBS card in a glassine bag to protect spots from chemicals in the desiccant and humidity indicator.

  Note: Protocols must define if the DBS card should be stored whole or cut into partitions for storage.
- ✓ Seal the card (or card fractions) in a gas-impermeable, zip-lock bag containing a desiccant pack and humidity indicator. Store no more than one card per bag.

  Note: Do not use electric dryer or oven to dry cards. Store no more than one DBS card per bag. Do not heat, stack or allow DBS to touch other surfaces during the drying process. Use a minimum of 3 desiccant packs per zip lock bag. Humidity cards may be recharged before use. If the humidity card is pink at the 30% level, you may recharge the indicator card and desiccant pack by heating at 50-60○C for 3-4 hours in a drying oven; then cool 10 minutes. IMMEDIATELY RETURN card and desiccant pack to sealable plastic bag.

E. **DBS Specimen Storage**

Variable storage conditions have been reported for DBS generated from Whatman 903 paper. In general, extreme temperatures (>37°C) in the presence of high humidity are not acceptable storage conditions because they promote microbial growth and alter elution of analytes off the paper. Stability of proteins and nucleic acids (HIV DNA, HIV RNA, and HBV DNA) have been demonstrated after storage in a refrigerator (2 to 8°C) or at room temperature (15 to 30°C) for up to 1 year. Stability of genotypic HIV drug resistance testing has been demonstrated after storage in a refrigerator (2 to 8°C) or at room temperature (15 to 30°C) for up to 12 weeks. However, for optimal preservation, long term storage (> 90days) at ≤ -20°C is recommended. Protocols must define the storage conditions for DBS based on the analyte being tested, the assay being used, and the paper used for collection.

- ✓ Store the DBS bag according to protocol MOPS (if available) and/or LPC until shipped to the processing or receiving laboratory. The protocol team will notify sites when to

ship samples to the receiving laboratory.

- Make sure the patient identifier and date (or LDMS generated label) are on the DBS Whatman Protein Saver 903 Card and on the outside of the bag, and that the bag is sealed.

Note: Labels affixed to the outside of the gas impermeable bags may fall off during storage in a freezer.

- Store DBS cards in sealed bags in a 2" fiberboard freezer box (regardless of the storage temperature) and inventory using the LDMS storage module.
- Store DBS cards in a clean, dry area of lab that has no exposure to direct sunlight, is free of insects and rodents, and where ambient temperatures will not exceed 30C.
- Check humidity indicators in bags weekly while DBS are stored at room temperatureor in a refrigerator. Change desiccant packs if indicator color changes from blue to pink. Document the date of any observed humidity changes on the humidity card and in the LDMS; add a new humidity indicator and leave the old and new humidity indicators in the bag. Make sure bags are sealed tightly.

F. **DBS Specimen Shipping**

✓ Ship DBS Whatman Protein Saver 903 Cards to a testing laboratory, or transfer the stored DBS cards to a freezer (≤ -20°C) within 3 months of collection(or as defined in the protocol).

✓ Shipping conditions:

- Check protocol for required shipping conditions to be used for shipping DBS samples to testing laboratories and/or to the specimen repository.
- DBS cards that are stored at room temperature (15 to 30°C) may be shipped under ambient conditions or on dry ice. Inspect DBS specimens for contamination or humidity change, and replace desiccants prior to shipping.
- DBS cards stored in a refrigerator may be shipped under ambient conditions or on dry ice, but the DBS cards must be equilibrated to room

temperature (minimum of 30 minutes) prior to shipping under ambient conditions; inspect DBS specimens for contamination or humidity change, and replace or add additional desiccants prior to shipping; document any changes in the humidity indicator in the LDMS.

- DBS cards stored at ≤ -20°C may be shipped on dry ice or under ambient conditions. If the frozen cards are to be shipped under ambient conditions, the DBS cards must be equilibrated to room temperature (minimum of 30 minutes) prior to shipping under ambient conditions; inspect DBS specimens for contamination or humidity change, and replace or add desiccants prior to shipping; may record this information in the LDMS.

✓ Ship DBS cards as non-dangerous goods (they are exempt biological specimens according to ICAO and IATA).

✓ If shipping specimen boxes:

- Secure boxes with a rubber band so they do not open during transit.
- Place boxes in a water tight secondary container (i.e. Tyvek® bags) to protect the specimens from humidity during transit.

Note: The box may be placed in a second ziplock bag with additional dessicants before placing into the Tyvek bag for shipping to provide additional protection against humidity.

- Tyvek® bags containing boxes may be placed in larger shipping envelope or box.

✓ If shipping individual specimen cards:

- Place individual specimen cards in water tight secondary containers (i.e. Tyvek® bags) to protect the specimens from humidity in transit.

Note: The individual specimen card may be placed in a second zip lock bag with additional dessicants before placing into the Tyvek bag for shipping to provide additional protection against humidity.

- Place the Tyvek® bags containing individual DBS cards in a shipping envelope (ambient) or into an approved container filled with dry ice (frozen). Follow shipping guidelines for dry ice shipments.

- ✓ Ship DBS cards by overnight or second-day courier services. Be sure to follow the ACTG/IMPAACT shipping instructions for ambient and/or dry ice shipments http://www.hanc.info/labs/labresources/procedures/Pages/actnShippingDemo.aspx
    - ACTG/IMPAACT Shipping Documents
    - Provide an LDMS Diskette or CD (place in disk/CD mailer).
    - Provide an LDMS shipping manifest for all boxes. Shipping manifest(s) must exactly match the label information and order in the associated shipment, including the global specimen IDs.
    - Provide an LDMS Box map for all boxes. Box maps must exactly match the label information and order in the associated shipment, including the global specimen IDs.
    - Provide any relevant CRFs. Confirm with receiving lab if CRFs are required. Do NOT send CRFs to the Repository.
- ✓ Courier Information and Notification
    - Record courier service on ACTG/IMPAACT Specimen Shipment Notice.
    - Record courier air bill number on ACTG/IMPAACT Specimen Shipment Notice.
    - Advance notification of shipment must be made to the recipient. The preferred method of notification is to FAX or email completed ACTG/IMPAACT Specimen Shipment Notice.
- ✓ **Examples of Invalid Spots and Invalid Collections of DBS**
    - Samples with variable blood volumes.

Sample Volume: 3 Large OK; ***1 Medium, 1 Small not usable***

- Spot with incomplete absorption resulting in insufficient blood volumes.

This specimen is invalid because quantity of blood is insufficient for testing. This may have been caused by the following:

- Removing filter paper before blood has completely filled circle or before blood has soaked through to the other side

- Applying blood to the DBS card with a capillary tube
- DBS paper coming in contact with ungloved hands or substances such as hand lotion or powder, either before or after blood specimen collection.

- Sample that was potentially rubbed against the child's heel

This specimen is invalid because it appears scratched or abraded.

- Sample not allowed to air dry before placing in storage bags

This specimen is invalid because the specimen was not dry before mailing. DBS must dry a minimum of 4 hours (preferably overnight, especially in areas with high humidity) before packaging and shipping.

- Sample where blood was clotted and did not soak into paper

This specimen is invalid because the specimen appears clotted or layered. The volume of specimen will not be uniform between spots resulting in errors during the testing process. This may have been caused by:

- Touching the same circle on the filter paper to blood drop several times.
- Filling circle on both sides of filter paper

- DBS where serum separated from cells

This specimen is invalid because the specimen exhibits serum rings i.e. serum becomes separate from cells. This may have been caused by the following:

- Not allowing alcohol to dry at puncture site before making skin puncture
- Allowing filter paper to come in contact with alcohol, hand lotion, etc
- Squeezing area surrounding puncture site excessively
- Drying specimen improperly

Applying blood to filter paper with a capillary tube

Environmental and safety controls

Universal precautions must be taken while collecting DBS specimens. Dried blood spots on filter paper are not considered to be a biohazard, but whole blood handled during specimen collection and reconstituted specimens obtained during processing may be hazardous. Appropriate personal protective equipment, including gloves and a lab coat/gown, should be worn at all times, to ensure safe handling of samples. If you should tear a glove, remove the torn one and replace it immediately. If a needle puncture should occur, follow your site/institution policy for handling work-related injuries.

Procedure

Elution of Dried Blood Spots

1. Punch out one spot with a single-use 6 mm device from each blood-soaked circle of the Grade 903 filter card. Transfer all punched dried blood spots from a single patient to one well of the 12-well plate.

2. Fill the well with phosphate-buffered saline containing 0.05% Tween 20 and 0.08% sodium azide. Adapt the volume of added buffer to the minimal respective requirements of the assay used for subsequent analysis of dried blood spots elutes.
3. Repeat these steps to obtain a second series of dried blood spot eluates in order to perform molecular analyses.
4. Put the cell culture plate on a laboratory shaker and let the punched dried blood spots gently elute for a minimum of 4 hr or, preferably, O/N.
5. The next day, the spots are almost free from blood and hemolytic supernatants have formed. Transfer these eluates to microcentrifuge tubes. Then, subject them to centrifugation for 2 min at 10,500 x g to free the supernatants from any debris that had formed during elution

Analysis of Eluates

Investigate the eluates for hepatitis C virus using commercially available kits and follow the respective manufacturers' instructions.

References

1. Coombs, Robert W Investigator, Principal Fiscus, Susan. Processing of Dried Blood Spots Processing of Dried Blood Spots Standard Operating ProcedureProcedure Table

of Contents,2012; page 1-20

2. Grüner, Nico Stambouli, Oumaima Ross, R Stefan. Dried Blood Spots - Preparing and Processing for Use in Immunoassays and in Molecular Techniques, 2015; march 1-9

Annex IV. Standard operating procedure tor rapid diagnostic test

Purpose	For the qualitative detection of antibodies to hepatitis C virus in human whole blood ,serum or plasma

Abbreviations	HVC= hepatitis C virus , HBV= hepatitis B virus HIV= human immunodeficiency virus

Materials

Reagents
1. Disposable gloves
2. HCV antibody test cassettes
3. Sample diluents (4ml) if applicable
4.Test tube

Supplies
1. Centrifuge
2. Test tube ruck
3. Timer

Sample	Sample type	Amount required	Transport Storage	Stability
	Serum, plasma, and whole blood	*o.2-2ml*	stored at 2-8 degree centigrade if the test is to be run within one week	stored at 2-8 degree centigrade if the test is to be run within one week

Safety Precautions

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens are being handled.
10. Dispose of all specimens and materials used to perform the test as bio hazardous Waste.

11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong Air-conditioning.

Procedure	**Step**	**Action**
	1	1. When you are ready to begin testing, open the sealed pouch by tearing along the notch. Remove the test kit from the pouch and use it as soon as possible.
	2	2. Following the illustration, dip the test strip with the arrow side pointing down into the vessel of serum for about 10 seconds. Do not immerse past the marker line. Take the strip out and lay it flat on a clean, dry and non-absorbent surface
	3	. Wait for 15 minutes and read results. It is important that the background is clear before the result is read. Do not read results after 30 minutes.

Result Interpretation	**Negative:** Only one color band appears on the control (C) region. No apparent band on the test (T) region. **Positive:** In addition to a pink colored control (C) band, a distinct pink colored band will also appear in the test (T) region. **Invalid:** A total absence of color in both (C) and (T) regions and no colored band appears on the control (C) region is an indication of procedure error and/or the test reagent has deteriorated. Repeat with a new test kit. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

LIMITATIONS

1. This test should be used for the detection of antibodies to HCV in serum samples.

2. Only detect the presence of Anti-HCV, it should not be used as the sole criteria for the diagnosis of Hepatitis C viral infection.

3. As with all diagnostic tests, all results must be considered with other clinical information available to the physician. A definite clinical diagnosis should only be made by the physician after all clinical and laboratory findings have been evaluated.

4. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result any time does not preclude the possibility of Hepatitis C Virus infection.

Principle	One Step ANTI-HCV Test is based on the principle of double antigen sandwich immunoassay for determination of anti-HCV in serum. Purified recombinant antigens are employed to identify anti-HCV specifically. This one step test is very sensitive and only takes 10-20 minutes for the result to be read. Test results are read visually without any instrument.

Clinical Utility	Hepatitis C virus (HCV) infection is a common cause of liver disease in thalassemia major patients. The relationships of the infection to blood transfusion and the infection's effects on liver function have also been determined

Reference

1. Engvall E, Perimann P. Enzyme-Linked Immunosorbent Assay (ELISA) Quantitative Assay of Immunoglobulin G. Immunochem 1971:8:871-4.
2. Engvall E.Perimann P. Enzyme-Linked Immunosorbent Assay (ELISA). In: Pecters H, editor. Protides of the Biological Fluids. Proceedings of the Nineteenth Colloquium, Burgge. Wxford: Pergamon Press, 1971:553-6.
3. Engvall E, lonsson K, Perimann P Enzyme-Linked mmunogolbulin G By Means of Enzyme-Labelled Antigen and Antibody-Coated Tubes. Biochem Biophys Acta 1971,
4. BanWeemen BK, Schuurs AHWM. Immunoassay Using Antigen-Enzyme Conjugates. FEBS Letters 1971:15:232- 6.
5 Wisdom GB. Enzyme-Immunoassay.Clin Chem 1976;22:1243-55.
6 .Wolters G, Kuijpers L, Kacaki 1, Schuurs A. Solid-Phase

Declaration

I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

M.Sc. candidate: **Mulukan Akalu(B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Dr. Mistre Wolde (MSc, PhD, AssociateProfessor)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Ms. Sosina Walelign(MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia