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College of Graduate studies

**Effect of Handling of Laboratory Wastes on Liver Function
Tests among Handlers in National Laboratory for Public
Health in Khartoum State**

تأثير التعامل مع النفايات المخبرية في وظائف الكبد لعمال النظافة بالمعمل القومى للصحة العامة
في ولاية الخرطوم

A Dissertation submitted in partial fulfillment for the requirement of M.sc. in medical laboratory
science (clinical chemistry)

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2015

الاية

بسم الله الرحمن الرحيم

(فَتَبَسَّمْ ضَاحِكًا مِنْ قَوْلِهَا وَقَالَ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ
وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ)
(19)

(سورة النمل)

Dedication

To my Father

To my Mother

To my Husband

To my family

To my friends

Acknowledgment

First I wish to thank Allah for granting me the Confidence and Success to complete this study.

I would like to express my sincere gratitude to my supervisor, Dr. Mariam Abass, for her advices and encouragement during this study.

We acknowledge each National laboratory for public health director and management for allowing us to undergo this study. We are also grateful to the leader of waste handlers, for data collectors and laboratory technicians, and the study subjects who kindly volunteered to participate in this study.

Finally my gratitude goes to my parent, and my husband who have been most supportive all the time.

Abstract

Viral hepatitis is an inflammation of the liver due to viral infections and there are groups of viruses that affect the liver of which hepatitis B and C viruses are the causative agents of severe form of liver disease with high rate of mortality. Laboratory waste handlers who undergo collection, transportation, and disposal of laboratory wastes in the National laboratory for public health are at risk of exposure to acquire those infections which transmit mainly as a result of contaminated blood and other body fluids including injury with sharp instruments, splash to the eye or mucous membrane. This study aimed to determine the prevalence of hepatitis B and/or C viruses and associated liver function tests among laboratory waste handlers.

This is a cross-sectional study was conducted in March 2015, in national laboratory for public health, among 50 workers after acquiring ethical clearance and informed consent data were collected by using pre-tested and well structured check list. Venous bloods were collected and the serums were tested for hepatitis B surface antigen and anti-hepatitis C antibody using Enzyme Linked Immuno sorbent Assay technique (ELISA) and automated machine (Hitachi) for measuring liver function tests. Data was entered and analyzed using SPSS software package (version 19).

The statistical analysis showed that: The 50 non vaccinated workers, an adequate awareness about type of waste among the group 48%, during work only 62% were wearing protective cloth and 84% were wearing gloves while handling laboratory waste, just about 24% had segregate the waste, and 18% used sharp container while handling sharpening. Only 8% had got pricked accidentally by handle needle. Only one worker (2%) had infected with HBV during last ten years, and no one had infected with HCV. When measuring LFT (total bilirubin, total protein,

albumin, alkaline phosphates, aspartate aminotransferase, and Alanine aminotransferase) for those workers, we found a significant decrease in total bilirubin, total protein, albumin, alkaline phosphates, aspartate aminotransferase. P. value prospectively (0.007, 0.000, 0.0003, 0.000, 0.026) and insignificant decrease for Alanine aminotransferas. P. value (0.435).

Overall, an inadequate awareness and a few risk perceptions about HBI were found among the study group with significant impairment in liver function test.

مستخلص الدراسة

التهاب الكبد الفيروسي هو التهاب الكبد الوبائي بسبب العدوى الفيروسية وهناك مجموعة من الفيروسات التي تؤثر على الكبد منها التهاب الكبد B و C ، الفيروسات هي العوامل المسببة للتعرض لأمراض الكبد الحادة مع ارتفاع معدل الوفيات. العاملون في المعمل القومي للصحة العامة الذين يقومون بعملية جمع ونقل والتخلص من النفايات المخبرية هم أكثر قابلية للتعرض لخطر تلك الأمراض التي تنقل بشكل رئيسي بواسطة الدم الملوث وسوائل الجسم الأخرى عن طريق الإصابة بأدوات حادة ، أو عن طريق دفعه إلى العين أو الأغشية المخاطية. هدفت هذه الدراسة إلى تحديد مدى انتشار التهاب الكبد B أو الفيروسات C وما يرتبط بها من اختبارات وظائف الكبد لعمال النظافة بالمختبر.

هذه دراسة مستعرضة أجريت في مارس 2015 ، في المعمل القومي للصحة العامة ، لعدد 50 عاملا بعد الحصول على موافقتهم المستنيرة جمعت البيانات اللازمة باستخدام قائمة لاختيار الإجابات المطابقة لكل فرد . وتم جمع الدماء الوريدية و إجراء اختبار وجود التهاب الكبد B و C باستخدام إنزيم مرتبط بالمناعي تقنية الفحص (ELISA) و الجهاز الآلي (هيتاشي) لقياس LFT . تم إدخال البيانات و تحليلها باستخدام حزمة البرامج (SPSS version 19) . وكان 50 من العمال غير المطعمين ، والوعي الكافي حول نوع من النفايات ضمن المجموعة 48 % ، خلال العمل فقط 62 % يرتدون الزى المخبري الواقى و كانت 84 % يرتدون القفازات أثناء التعامل مع النفايات المخبرية ، كان فقط حوالي 24 % يقومون بعملية تصنيف النفايات المخبرية ، و 18 % يستخدمون الحاويات الخاصة للأدوات الحادة أثناء التعامل مع الأدوات الحادة . وكان 8 % فقط قد تعرضوا لوخز عن طريق الخطأ عند التعامل مع الإبر. وكان عامل واحد فقط (2 %) تعرض للإصابة بمرض الكبد الفيروسي HBV خلال السنوات العشر الماضية، و ولا توجد إصابة بمرض الكبد الفيروسي HCV.

عند قياس LFT (البيليروبين الكلي، البروتين الكلي ، الزلال ، والفوسفات القلوية، و الألائين اسبارتاتي ، الأئين الألائين) لأولئك العمال، وجدنا تأثير واضح على (البيليروبين الكلي، البروتين الكلي ، الزلال ، والفوسفات القلوية، و الألائين اسبارتاتي). قيمة p value على التوالي (0.007 ، 0.000 ، 0.0003 ، 0.000 ، 0.026) و ولا يوجد تأثير على (alt) . قيمة p value (0.435) .

نلاحظ إن نسبة الوعي غير كاف ونسبة الإصابات بسيطة لالتهاب الكبد الفيروس (HBI) بين هذه المجموعة تحت الدراسة. وتغيير واضح في نتائج وظائف الكبد.

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List of Abbreviations

ALP	<u>Alkaline phosphatase</u>
<u>ALT</u>	Alanine transaminase
<u>AST</u>	Aspartate aminotransferase
<u>COSHH</u>	Control of Substances Hazardous to Health Regulations
ELISA	Enzyme Linked Immuno sorbent Assay
EPA	Environmental Protection Agency
GGT	Gamma-glutamyl transpeptidase
HBI	Hepatitis B infection
HBV	Hepatitis B virus
HCV	Hepatitis C virus
INR	International normalized ratio
ISO	International Organization for Standardization's
LFT	Liver Function Test
MPW	Medical Pathological Waste
MSDS	Material safety data sheets
MWHs	Medical waste handlers
OSHA	Occupational Safety and Health Administration
PT	<u>prothrombin time</u>
PTT	partial thromboplastin time
SGOT	Glutamate oxaloacetate transaminase
SGPT	Glutamate pyruvate transaminase
WHO	World Health Organization

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Chapter One

1. Introduction and literature review

1.1. Introduction

Clinical laboratories are significant generators of infectious waste, including microbiological materials, contaminated sharps, and pathologic wastes such as blood specimens and blood products. Most waste produced in laboratories can be disposed of in the general solid waste stream ([Mehrdad Askarian1](#). et al, 2012).

There are different types of medical waste management systems in different countries. Although, medical waste disposal options are not completely risk-free, the risks can be minimized with care. Improper disposal of medical waste including mixing general wastes with infectious wastes and improper handling may include damage to humans by sharp instruments, diseases transmitted to humans by infectious agents, therefore, proper management of medical waste is a subject of major concerns for a healthy environment (Mmanzural. et al ,2008).

The initial responsibility for implementing this management trained laboratory personnel. They are responsible for evaluating hazards, providing information necessary to make an accurate waste determination, and assisting in the evaluation of appropriate strategies for management, minimization, and disposal ([National Academies](#), 2011).

Health and safety in clinical laboratories is becoming an increasingly important subject as a result of emergence of highly infectious diseases such as Hepatitis ([Ajaz Mustafa](#). et al, 2008). Hepatitis B infection (HBI) is one of the major public health problems globally and is the 10th leading cause of death ([Shankargouda Patil](#). et al, 2013). Also HCV it can cause severe medical outcomes such as acute hepatitis, chronic liver diseases and hepatocellular carcinoma. Worldwide

information on the spread of infection resulting from waste handling is limited ([Yitayal Shiferaw](#). et al, 2011). Studies from developed countries have shown that occupational exposure to waste may result in HBV infection (WHO, 2003).

No data about the prevalence of HBV or effect on liver function test among laboratory waste handlers is available in Sudan. Therefore; this study was conducted to describe the prevalence of HBV infection and the effect on liver function test, among laboratory waste handlers in National laboratory for public health.

The study was conducted to study prevalence of hepatitis B virus among medical waste handlers in Addis Ababa, Ethiopia in only three hospitals among 126 MWHs. The result show that From 126 Medical waste Handlers (MWHs), HBsAg was detected in 8 (6.3%) and, and Anti-HBcAg in 60 (47.6%) ([Yitayal Shiferaw](#). et al, 2011).

Sudan is classified among the countries with high hepatitis B virus seroprevalence. Exposure to the virus varied from 47%–78%, with a hepatitis B surface antigen prevalence ranging from 6.8% in central Sudan to 26% in southern Sudan. [Hatim MY Mudawi](#)(2008). Previous findings have indicated that the positivity for HBsAg in Sudan is 16%–20%. Other study, among 245 participants selected from 3 hospitals, (Khartoum hospital, Khartoum north, and Omdurman hospital) 12 (4.9%) tested positive (Elduma and Saeed, 2011).

Prolong handling of laboratory waste may affect on liver function test .The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified through processes such as amino acid deamination, which produces urea. In conjunction with the spleen it is involved in the destruction of spent red blood cells and the reclamation of their constituents. It is responsible for

synthesizing and secreting bile and synthesizing lipoproteins and plasma proteins, including clotting factors. It maintains a stable blood glucose level by taking up and storing glucose as glycogen (glycogenesis), breaking this down to glucose when needed (glycogenolysis) and forming glucose from noncarbohydrate sources such as amino acids (gluconeogenesis) ([Edoardo G. Giannini](#). et al, 2005).

1.2. Literature review

1.2.1. Hazardous waste

A hazardous waste is a waste with a chemical composition or other properties that make it capable of causing illness, death, or some other harm to humans and other life forms when mismanaged or released into the environment (Solid Waste and Emergency Response, 2005).

Laboratory waste consisting of discarded cultures and stocks of infectious agents and associated microbiological; pathological wastes; selected isolation wastes; used and unused discarded sharps; human blood, or blood products; and other wastes defined:

- **Blood and blood products:** Discarded waste human blood and blood components, including serum and plasma, and materials containing free-flowing blood and blood components.
- **Cultures and stocks:** Discarded cultures and stocks of infectious agents and associated microbiological, including human and animal cell cultures from medical and pathological laboratories, waste from the production of biological, discarded live and attenuated vaccines, and culture dishes and devices used to transfer, inoculate, or mix cultures of infectious agents.
- **D. Pathological wastes:** Discarded pathological waste, including human tissues, organs, and body parts removed during surgery, autopsy, or other medical procedures.
- **Selected isolation waste:** Discarded waste material that is contaminated with excretions, exudates, and secretions from patients with highly communicable diseases, and that is treated in isolation, includes blood and blood components, and sharps.

- **Sharps:** Discarded implements or parts of equipment used in animal or human patient care, medical research, or industrial laboratories, including hypodermic needles; syringes, Pasteur pipettes, scalpel blades, blood vials, needles with attached tubing, broken or unbroken glassware that has been in contact with an infectious agent, slides, cover slips, and unused, discarded implements or parts of equipment see medical waste disposal policy (1991).
- **Radioactive wastes:** are [wastes](#) that contain [radioactive](#) material. Radioactive wastes are usually [by-products](#) of [nuclear power](#) generation and other applications of [nuclear fission](#) or [nuclear technology](#), such as [research](#) and [medicine](#). Radioactive waste is [hazardous](#) to most forms of life and the environment, and is [regulated](#) by government agencies in order to protect human health and the environment. Joint Convention on the Safety of Spent Fuel Management and on the Safety of Radioactive Waste Management (2014).
- **Chemical waste:** is a [waste](#) that is made from harmful chemicals. Chemical waste may fall under regulations such as Control of Substances Hazardous to Health Regulations [COSHH](#) in the [United Kingdom](#), or the [Clean Water Act](#) and [Resource Conservation and Recovery Act](#) in the [United States](#). In the U.S., the Environmental Protection Agency (EPA) and the Occupational Safety and Health Administration (OSHA), as well as state and local regulations also regulate chemical use and disposal. Chemical waste may or may not be classed as [hazardous waste](#). Hallam, Bill (2010).

It considers to be hazardous if it has properties one of the following:

Toxic, corrosive (e.g.: acid PH <2 or basis PH >12), flammable, Reactive. Zaimastura BintiIbrahim (2005).

1.2.1.1. Laboratory awareness of handling different types of waste

These wastes must be handled carefully to prevent personal injury and the spread of disease (Charles Calmbacher, 2011).

Biohazard waste must be placed in waste containers with the biohazard symbol on them. Often facilities will use a color-coding system (e.g., red bags or red containers) to delineate biohazard trash. If a red trash container is used, the container as well as the bag inside must have the biohazard symbol. The symbol can be black on a red bag or container or orange-red on any other colored bag or container. Some autoclavable bags are clear with the symbol in black or red-orange.

The International Organization for Standardization's ISO 15190 recommends autoclaving of all microbiological specimens before they are placed into the waste stream as trash. Once autoclaved, they can go out as regular trash as they are no longer considered infectious.

Sharps containers can be made of anything as long as they are leak-proof on the sides and bottom and puncture resistant. Keep sharps containers upright throughout use. Check containers routinely and dispose of them when three-quarters full. OSHA does not require that sharps containers be puncture-proof, only puncture resistant. Sharps containers must have the biohazard symbol prominently displayed on the container.

Chemical waste must be handled according to the manufacturer's requirements on the material safety data sheets (MSDS). Hazardous chemical disposal is regulated through state and local governmental agencies. It is important to get proper authorization from these agencies to dispose of the chemicals and utilize licensed lab-packing companies to package and ship the chemical waste for proper disposal.

Regulated waste is placed in containers that are closable and constructed to contain all contents and to prevent fluids from leaking during handling, storage, transport, or shipping. The containers are labeled and closed before removal. If outside contamination of the regulated waste container occurs, it is placed in a second container that has the same qualities as the first (e.g., closable, leak-proof) and is handled in the same manner (Terry Jo Gile, 2011).

1.2.1.2. Segregation of waste

Wastes should be segregated at the point of generation according to their types:

- Infectious, bio-contaminated wastes (including Blood, sharp materials);
- Chemical wastes (chemical solutions, Powders, Chemical reagents etc.....
- Non infectious, common wastes (paper, cardboard, glass, or the like; empty chemical product containers should be treated as chemical wastes) and the household waste.
- Radioactive waste.
- Remains of Human tissues and Biopsies by Dr. Abdel Salam Ibrahim Bashir

1.2.1.3. TREATMENT OF WASTES

The goals of biomedical waste treatment are to reduce or eliminate the waste's hazards, and usually to make the waste unrecognizable. Treatment should render the waste safe for subsequent handling and disposal. There are several treatment methods that can accomplish these goals.

Biomedical waste is often incinerated. An efficient incinerator will destroy pathogens and sharps. An autoclave may also be used to treat biomedical waste. An autoclave uses steam and pressure to sterilize the waste or reduce its microbiological load to a level at which it may be safely disposed of. Many healthcare facilities routinely use an autoclave to sterilize medical supplies. If the same autoclave is used to sterilize supplies and treat biomedical waste,

administrative controls must be used to prevent the waste operations from contaminating the supplies. Effective administrative controls include operator training, strict procedures, and separate times and space for processing biomedical waste. For liquids and small quantities, a 1-10% solution of [bleach](#) can be used to disinfect biomedical waste. Solutions of sodium hydroxide and other chemical disinfectants may also be used, depending on the waste's characteristics. Other treatment methods include heat, [alkaline digesters](#) and the use of microwaves (WHO, 2007).

1.2.1.4. Disposal of laboratory waste

- **TISSUE CULTURE WASTE:** WASTE SHOULD BE AUTOCLAVED BEFORE BEING PLACED IN YELLOW BAGS AND SENT FOR INCINERATION.
- **Sharps;** Syringe needles, blades and disposable sharp instruments. Use only those yellow containers supplied via bulk stores for the disposal of sharps. Wherever possible, use safety devices for the removal of needles and blades from handles, or use disposables. Sharps containers must be properly and securely assembled by following the manufacturer's instructions. Before the contents reach the top seal the bin and label it with the following information: Date, contact telephone number, description of contents and departmental reference code. Never put an individuals' name on the label ([Statement of Health and Safety Policy and Statement of Organization and Arrangements, 2007](#)).
- **Biohazardous solid waste** typically consists of items such as contaminated gloves, paper towels, plastic ware and agar. Small volumes of liquid waste may also be included provided that any liquids are sealed in plastic tubes; of no greater than 50 mL volume and that the total aggregate volume per biohazard bag/bin does not exceed 500 mL. Biohazardous solid

waste may be disposed of either by decontamination off-site by the approved external contractor or on-site by autoclave sterilisation. Solid waste **MUST** be placed into a yellow biohazard bag that is lined with a paper kleensac (this helps prevent puncturing or tearing of the plastic bag). When in use (i.e. the bag is open) the biohazard bag and liner should be contained within a solid-sided bin with a lid (these bins may also be used to transport waste through non-laboratory areas). Biohazard bags must be filled no higher than 2/3 full, sealed with a cable tie or tape, then labelled with the department/area the waste originates from (for laboratory waste, also label with the lab group/room number) (University of Otago, 2011).

- **Pathological waste** must be packaged separately from other Medical Pathological Waste(MPW) to avoid odors and sanitation problems

1. Fold the flaps down on the outside of the box.
2. Place TWO plastic bags (one inside the other) into the box and pull the bag tops down over the flaps.
3. A filled MPW box should weigh **NO MORE** than 40 pounds or be no more than 3/4 full.
4. Close each bag separately. Twist plastic bag at the top; bend the twisted portion to form a loop and seal using the plastic bag closure tie.
5. Close the box.
6. Print your building, room number, and waste type (pathological or non-pathological) on box top label area. (National institute of health, 2014).

All human anatomical waste should be disposed by buried or incineration (Prudent practices for handling and disposal of infectious material, 1989).

- **Radioactive waste** includes solid, liquid and gaseous material contaminated with radionuclide. It produced as the result of procedure such as in vitro

analysis of body tissue and fluid this waste usually with short half –life which loses their activity relatively quickly. Zaimastura BintiIbrahim (2005).

1.2.2. The liver

The liver is an important organ of the body that is responsible for detoxification, metabolism, synthesis and storage of various substances. It's the largest internal organ in the body (the skin is considered the largest organ in the entire body) and it weighs about 3 pounds ([Charles](#), 2014). It is also a gland because it secretes chemicals that are used by other parts of the body (Benjamin, 1996).

The liver is a reddish brown triangular organ with four [lobes](#) of unequal size and shape. A human liver normally weighs 1.44–1.66 kg (Cotran and Ramzi, 2005).

It is both the largest internal organ and the largest [gland](#) in the human body. Located in the [right upper quadrant](#) of the [abdominal cavity](#), it rests just below the [diaphragm](#), to the right of the stomach and overlying the [gallbladder](#). It is connected to two large [blood vessels](#), the [hepatic artery](#) and the [portal vein](#). The hepatic artery carries oxygen-rich blood from the aorta, whereas the portal vein carries blood rich in digested nutrients from the entire [gastrointestinal tract](#) and also from the [spleen](#) and [pancreas](#) (Dorland, 2012).

1.2.2.1. The functions of the liver

The liver has multiple functions. It makes many of the chemicals required by the body to function normally, it breaks down and detoxifies substances in the body, and it also acts as a storage unit. Hepatocytes (hepar=liver + cyte=cell) are responsible for making much of the proteins in the body that are required for protein synthesis, including blood clotting factors, and albumin, required to maintain fluid within the circulation system. The liver is also responsible for

manufacturing [cholesterol](#) and [triglycerides](#). Carbohydrates are also produced in the liver and the organ is responsible for converting [glucose](#) into glycogen that can be stored both in the liver and in the muscle cells. The liver also makes bile that helps with food digestion.

The liver plays an important role in detoxifying the body by converting ammonia, a byproduct of metabolism in the body, into urea that is excreted in the urine by the kidneys. The liver also breaks down medications and drugs, including alcohol, and is responsible for breaking down [insulin](#) and other hormones in the body.

The liver is also able to store vitamins and chemicals that the body requires as building blocks. This includes: vitamin B12, folic acid, iron required to make red blood cells, vitamin A for vision, vitamin D for calcium absorption, and Vitamin K to help blood to clot properly (Benjamin, 1996).

1.2.2.2. DISORDERS OF LIVER

- Parasites and viruses can infect the liver, causing inflammation and that reduces liver function. The viruses that cause liver damage can be spread through blood or semen, contaminated food or water, or close contact with a person who is infected. The most common types of liver infection are hepatitis viruses, including: Hepatitis A, B, C, D, or E.

AN ABNORMAL GENE INHERITED FROM ONE OR BOTH PARENTS CAN CAUSE VARIOUS SUBSTANCES TO BUILD UP IN LIVER, RESULTING IN LIVER DAMAGE. GENETIC LIVER DISEASES INCLUDE:

- Hemochromatosis (is a disease in which deposits of iron collect in the [liver](#) and other organs).

- Wilson's disease (copper metabolism disorder that leads to excessive buildup of copper in the liver. It results from an inherited genetic mutation and can cause liver, brain, and kidney damage ([Mayo Clinic Staff](#), 2014).
- [Cirrhosis](#) (is a [chronic](#) liver disease that causes advanced scarring of liver tissue. The liver can repair many injuries, but cirrhosis is usually irreversible). [Nonalcoholic fatty liver disease](#) and [nonalcoholic steatohepatitis](#), (both associated with the accumulation of fat in the liver).
- ALCOHOLIC LIVER DISEASE: THE LIVER IS RESPONSIBLE FOR THE METABOLISM OF ALCOHOL. IT BREAKS IT DOWN SO THAT IT CAN THEN BE ELIMINATED FROM THE BODY. ALCOHOL CAN DAMAGE OR DESTROY LIVER CELLS, AND THE LIVER CAN BE SERIOUSLY INJURED WHEN MORE ALCOHOL IS CONSUMED THAN IT CAN PROCESS. EXCESSIVE CONSUMPTION OF ALCOHOL IS A COMMON CAUSE OF LIVER DAMAGE.
- OBSTRUCTION OF BILE DUCTS, GALLSTONES, [TUMORS](#), TRAUMA, [CYSTS](#), AND [INFLAMMATION](#) CAN CAUSE BLOCKAGES OR OBSTRUCTIONS IN THE BILE DUCTS. WHEN THIS IS SEVERE, [BILE](#) AND ITS ASSOCIATED WASTES ACCUMULATE IN THE LIVER, CAUSING [JAUNDICE](#), DARK BROWN URINE, AND PALE-COLORED STOOLS.
- [Autoimmune disorders](#) are diseases caused by an inappropriate immune response against the body's own tissues. Sometimes these conditions can affect the liver.

1.2.2.3.Signs and symptoms of liver disease include: Skin and eyes that appear yellowish (jaundice), abdominal pain and swelling, swelling in the legs and ankles, itchy skin, dark urine color, pale stool color, or bloody or tar-colored stool, chronic fatigue, nausea or vomiting, loss of appetite and tendency to bruise easily.

1.2.2.4. The Liver function tests:

LIVER FUNCTION TESTS (LFTs OR LFs) ARE GROUPS OF BLOOD TESTS THAT GIVE INFORMATION ABOUT THE STATE OF A PATIENT'S [LIVER](#) (LEE AND MARY, 2009).

These tests include [prothrombin time](#) (PT/INR), albumin, bilirubin (direct and indirect), Liver transaminases ([AST](#) or SGOT and [ALT](#) or SGPT) are useful biomarkers of liver injury in a patient with some degree of intact liver function. And some with conditions linked to the biliary tract ([gamma-glutamyl transferase](#) and [alkaline phosphatase](#)), and others (Mengel. et al, 2005).

Total protein: Reference range: 6.0 to 8.0g/dl. This measures albumin and all other proteins in blood.

Albumin: Reference range: 3.5 to 5.3 g/dl. [Albumin](#) is a protein made specifically by the liver, and can be measured cheaply and easily. It is the main constituent of total protein (the remaining from [globulins](#)). Albumin levels are decreased in chronic liver disease, such as [cirrhosis](#). It is also decreased in [Nephrotic syndrome](#), where it is lost through the urine. The consequence of low albumin can be edema since the intravascular [oncotic pressure](#) becomes lower than the extravascular space. An alternative to albumin measurement is prealbumin, which is better at detecting acute changes (half-life of albumin and prealbumin is about 2 weeks and about 2 days, respectively) (Nyblom. et al, 2004; Nyblom. et al , 2006).

Alanine transaminase (ALT). Reference range (up to 40 IU/L). This is an enzyme that helps to process proteins. (An enzyme is a protein that helps to speed up chemical reactions.it is part of the normal metabolic processes in the liver and is responsible for transferring amino acids (components that build proteins) from one molecule to another ([Siamak](#), 2014). Various enzymes occur in the cells in the

body. Large amounts of ALT occur in liver cells. When the liver is injured or inflamed (as in hepatitis), the blood level of ALT usually rises.

Aspartate aminotransferase (AST). Reference range (6-40 IU/L). This is another enzyme usually found inside liver cells. It is also part of the normal metabolic processes in the liver and is responsible for transferring amino acids (components that build proteins) from one molecule to another ([Siamak, 2014](#)). When a blood test detects high levels of this enzyme in the blood it usually means the liver is injured in some way. However AST can also be released if heart or skeletal muscle is damaged. For this reason ALT is usually considered to be more specifically related to liver problems. (www.patient.co.uk/health/liver-function-tests.)

Alkaline phosphatase: Reference range (44 to 147 IU/L). Alkaline phosphatase (ALP) is a protein found in all body tissues. Tissues with higher amounts of ALP include the liver, [bile](#) ducts, and bone. Abnormal results may be due to the following conditions:

Higher-than-normal ALP levels: [Biliary obstruction](#) , Bone conditions ,[Osteoblastic bone tumors](#), [osteomalacia](#), a fracture that is healing ,Liver disease or [hepatitis](#), Eating a fatty meal , if you have blood type O or B, [Hyperparathyroidism](#) ,Leukemia , Lymphoma , [Paget's disease](#), [Rickets](#) , Sarcoidosis .

Lower-than-normal ALP levels: Hypophosphatasia, Malnutrition, [Protein](#) deficiency and Wilson's disease (Afdhal. et al, 2013; Berk. et al, 2013; Martin, 2013).

Gamma-glutamyl transpeptidase: Reference range (0 to 42 IU/L).

Gamma- glutamyl transpeptidase (GGT) is a test to measure the amount of the [enzyme](#) GGT in the blood. This test is used to detect diseases of the liver or [bile](#) ducts. It is also done with other tests (such as the [ALT](#), [ALP](#), and [bilirubin](#) tests) to tell the difference between liver or bile duct disorders and bone disease. It may also be done to screen for or monitor alcohol abuse.

Greater-than-normal levels of GGT may be due to: Alcohol abuse , Diabetes , Flow of bile from the liver is blocked ([cholestasis](#)) , [Heart failure](#) , [Hepatitis](#) , Liver ischemia (lack of blood flow), Liver tumor , Lung disease , Pancreas disease , Scarring of the liver ([cirrhosis](#)) , Use of drugs that are toxic to the liver (Berk. et al , 2013; Feldman. et al, 2013).

Bilirubin: Reference range (0.1 to 1.0mg/dl). Measurement of total [bilirubin](#) includes both unconjugated and conjugated bilirubin. Unconjugated bilirubin is a breakdown product of [heme](#) (a part of [hemoglobin](#) in red blood cells). It is mainly transported bound to albumin circulating in the blood. Addition of high-concentration hydrophobic drugs (certain antibiotics, diuretics) and high free fatty acids can cause elevated unconjugated bilirubin. Heme can also come from myoglobin, found mostly in muscle, cytochromes, found mostly in mitochondria, catalase, peroxidase, and nitric oxide synthase. The liver is responsible for clearing the blood of unconjugated bilirubin, and about 30% of it is taken up by a normal liver on each pass of the blood through the liver by the following mechanism: bilirubin is taken up into [hepatocytes](#), 'conjugated' (modified to make it water-soluble) by [UDP-glucuronyl-transferase](#), and secreted into the [bile](#), which is excreted into the intestine. In the intestine, conjugated bilirubin may be metabolized by colonic bacteria, eliminated, or reabsorbed. Metabolism of

bilirubin into [urobilinogen](#) followed by reabsorption of urobilinogen accounts for the yellow color of urine, as urine contains a downstream product of urobilinogen. Further metabolism of urobilinogen into stercobilin while in the bowels accounts for the brown color of stool. Thus, having white or clay-colored stool is an indicator for a blockage in bilirubin processing and thus potential liver dysfunction or cholestasis.

Increased total bilirubin (TBIL) causes [jaundice](#), and can indicate a number of problems:

- i. Prehepatic: Increased bilirubin production can be due to a number of causes, including [hemolytic anemias](#) and internal hemorrhage.
- ii. Hepatic: Problems with the liver are reflected as deficiencies in bilirubin metabolism (e.g., reduced hepatocyte uptake, impaired conjugation of bilirubin, and reduced hepatocyte secretion of bilirubin). Some examples would be cirrhosis and viral hepatitis.
- iii. Posthepatic: Obstruction of the bile ducts is reflected as deficiencies in bilirubin excretion. (Obstruction can be located either within the liver or in the [bile duct](#)) (Pincus. et al, 2012).

Blood clotting tests (PT and INR): Besides its functions in [metabolism](#), the liver makes proteins that are essential for normal blood clotting. Sometimes additional tests are performed to help establish the liver's ability to make these proteins:

- Prothrombin time (PT): A test of the time it takes for a blood sample to clot, under specific conditions in a laboratory. If low levels of clotting factors are present, the prothrombin time is longer.

- International normalised ratio (INR): Not a test, but a standardised way for all laboratories to report PT, so their results can be compared accurately with each other.

PT and INR rise in people with severe liver disease because the liver fails to make normal amounts of certain clotting factors. An elevated PT can have many other causes besides liver disease, however. PT is often checked together with PTT (partial thromboplastin time). If PT and/or PTT are elevated, a problem with bleeding or clotting may be present (Feldman. et al, 2010).

Immunology: Blood tests may be done to detect:

- Viruses and antibodies to viruses. Various viral infections can cause hepatitis (inflammation of the liver) - for example, hepatitis A virus, hepatitis B virus, etc.
- Auto-antibodies. These are antibodies which attack a part of your own body and occur in
- Autoimmune disorders. The most common autoimmune disorders of the liver are:
 1. Primary biliary cirrhosis (associated with anti-mitochondrial antibodies).
 2. Autoimmune hepatitis (associated with smooth muscle antibodies).

Other types of protein in the blood can point to specific liver diseases - for example:

- Ceruloplasmin is reduced in Wilson's disease.
- Lack of 1-antitrypsin is an uncommon cause of cirrhosis.
- A high level of ferritin is a marker of haemochromatosis.
(www.patient.co.uk).

1.2.3.Hepatitis is a [medical condition](#) defined by the inflammation of the [liver](#) and characterized by the presence of [inflammatory cells](#) in the [tissue](#) of the organ. Hepatitis may occur with limited or no symptoms, but often leads to [jaundice](#) (a yellow discoloration of the [skin](#), [mucous membrane](#), and [conjunctiva](#)), [poor appetite](#), and [malaise](#). Hepatitis is acute when it lasts less than six months and chronic when it persists longer, Hepatitis viruses are the most common cause of hepatitis in the world, There are 5 main hepatitis viruses, referred to as types A, B, C, D and E. These 5 types are of greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread. In particular, types B and C lead to chronic disease in hundreds of millions of people and, together, are the most common cause of liver cirrhosis and cancer (WHO, 2014).

1.2.3.1. Hepatitis B virusHBV is a DNA virus classified in the virus family Hepadnaviridae. Humans are the only known natural host.HBV enters the liver via the bloodstream, and replication

Occurs only in liver tissue. The intact, infectious virus is 42–47 nm in diameter and circulates in the blood in concentrations as high as 10⁸ virions per ml. The inner core of the virus contains hepatitis B core antigen, hepatitis B e antigen (HBeAg), a partially double-stranded 3,200-nucleotide DNA molecule, and DNA polymerase with reverse transcriptase activity. Hepatitis B surface antigen (HBsAg) is found both on the surface of the virus and as self-assembling, noninfectious spherical or tubular particles (Colin.et al, 2006).

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus. It is a major global health problem. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer, Transmission of hepatitis B virus can survive outside the body for at least 7 days. During this time,

the virus can still cause infection if it enters the body of a person who is not protected by the vaccine. The incubation period of the hepatitis B virus is 75 days on average, but can vary from 30 to 180 days. The virus may be detected within 30 to 60 days after infection and can persist and develop into chronic hepatitis B. In highly endemic areas, hepatitis B is most commonly spread from mother to child at birth (perinatal transmission), or through horizontal transmission (exposure to infected blood), especially from an infected child to an uninfected child during the first 5 years of life. The development of chronic infection is very common in infants infected from their mothers or before the age of 5 years.

Hepatitis B is also spread by percutaneous or mucosal exposure to infected blood and various body fluids, as well as through saliva, menstrual, vaginal, and seminal fluids. Sexual transmission of hepatitis B may occur, particularly in unvaccinated men who have sex with men and heterosexual persons with multiple sex partners or contact with sex workers. Infection in adulthood leads to chronic hepatitis in less than 5% of cases. Transmission of the virus may also occur through the reuse of needles and syringes either in health-care settings or among persons who inject drugs. In addition, infection can occur during medical, surgical and dental procedures, tattooing, or through the use of razors and similar objects that are contaminated with infected blood (Lozano R. et al, 2010).

Acute hepatitis B for newly infected persons who develop acute hepatitis, the average incubation period (time from exposure to onset of jaundice) is 90 days (range: 60–150 days). The likelihood of developing symptoms of hepatitis as a result of a new HBV infection is age-dependent. Over 90 percent of perinatal HBV infections are asymptomatic, while the typical manifestations of acute hepatitis are noted in 5–15 percent of newly infected young children (1–5 years of age) and in 33–50 percent of older children, adolescents, and adults (7). Persons with acute

hepatitis B can show signs and symptoms that include nausea, abdominal pain, vomiting, fever, jaundice, and dark urine, changes in stool color, and hepatomegaly or splenomegaly (Colin. et al, 2006).

Chronic HBV infection is defined as either the presence of HBsAg in the serum for at least 6 months or the presence of HBsAg in a person who tests negative for immunoglobulin M antibodies to hepatitis B core antigen (Colin.et al, 2006).

Most of those with chronic disease have no symptoms; however, [cirrhosis](#) and [liver cancer](#) may eventually develop (Chang, 2007).

Vaccination protocol for hepatitis B: The vaccination schedule most often used has been three intramuscular injections. The following timing of injections is:

1 st dose	at elected date.
2 nd dose	4 – 10 weeks after the 1 st dose.
3 rd dose	1 – 5 months after the 2 nd dose.

Dosage and administration:

Adult dose vaccine: 20µg dose (1.0 ml suspension).

Booster dose:

It would seem advisable to recommend a booster dose when the anti-HBs anti body titer fall below 10 IU/L, particularly for all people at risk ([Shankargouda](#), 2013).

1.2.3.2. Hepatitis C virus: The hepatitis C virus is an RNA virus that belongs to the family flaviviridae. HCV replicates in the cytoplasm of hepatocytes, but is not directly cytopathic. Persistent infection appears to rely on rapid production of virus

and continuous cell-to-cell spread, along with a lack of vigorous T-cell immune response to HCV antigens. The HCV turnover rate can be quite high with replication ranging between 10^{10} to 10^{12} virions per day, and a predicted viral half-life of 2 to 3 hours (Stephen. et al, 2006). The rapid viral replication and lack of error proofreading by the viral RNA polymerase are reasons why the HCV RNA genome mutates frequently (Bukh . et al, 1995).

Hepatitis C is usually spread by blood-to-blood contact (when blood from a person with Hepatitis C contacts (touches or gets into) another person's [bloodstream](#)). The most common ways that this happens are through intravenous drug use (when a person shoots drugs into one of their veins, with a needle that was already used by a person infected with Hepatitis C); nonsterile medical equipment (medical tools that were not cleaned well enough after being used on an infected person); and [blood transfusions](#) (when a person is given blood that came from an infected person) (Houghton , 2009).

Eighty percent (or 8 out of every 10) of people exposed to the Hepatitis C virus get a chronic infection (Nelson. et al, 2011). Most experience very few or no symptoms during the first decades of the infection (Springer Verlag, 2011). But in people who have been infected for many years, Hepatitis C can cause serious problems, like cirrhosis and liver cancer (Rosen, 2011).

1.2.3.3. Prevention of hepatitis:

Passive Immunization: The discovery that passively acquired anti-HBs could protect individuals from acute clinical hepatitis B and chronic HBV infection if given soon after exposure led to the development of a specific Ig containing high titer of anti-HBs (HBIG). HBIG is prepared by the Cohn Oncly fractionation procedure from serum containing high titers of anti-HBs and is standardized to

100,000 IU of anti-HBs/ml. HBIG is effective, often in combination with hepatitis B vaccine, as postexposure prophylaxis following (i) perinatal exposure for an infant born to an HBsAg-positive mother, (ii) percutaneous or mucous membrane exposure to HBsAg-positive blood, or (iii) sexual exposure to an HBsAg-positive person.

Active immunization: Safe, immunogenic, and effective hepatitis B vaccines have been commercially available in the United States since 1981. Hepatitis B vaccines are composed of highly purified preparations of HBsAg. The vaccines are prepared either by harvesting HBs Ag from the plasma of persons with chronic infection (plasma-derived vaccine) or by inserting plasmids containing the HBs gene, and in some cases the pre-S1 and/or pre-S2 gene, into yeast or mammalian cells. The vaccines undergo various inactivation steps, are highly purified, and then are adjuvanted with aluminum phosphate or aluminum hydroxide and preserved with thimerosal. In the United States, vaccines are licensed for all age groups as a three-dose series consisting of two priming doses given 1 month apart and a third dose given 5 months after the second dose. The recommended dose varies with the product, the recipient's age, and, for infants, the mother's HBsAg serologic status. In general, the vaccine dose for infants and adolescents is 50 to 75% lower than that for adults ([Francis, 1999](#)).

1.3. Rationale

Laboratory wastes contain a wide range of microorganisms among which hepatitis B virus (HBV) are the most significant pathogens (Yitayal. et al, 2011). Hepatitis is an inflammation of the liver, most commonly caused by a viral infection. Of these viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for a substantial proportion of liver diseases worldwide. These viruses are responsible for liver damages ranging from minor disorders to liver cirrhosis and hepatocellular carcinoma. [Abel Girma Ayele](#) and [Solomon Gebre-Selassie](#), 2012.

Knowledge of handling of waste may have serious health consequence (hepatitis or liver impairment).

Since no data about the prevalence of HBV and affect on liver function test among laboratory waste handlers is available in Sudan. The present study aimed to investigate the prevalence of HBV, HCV or decrease in liver function for laboratory handlers for HBV and HCV for 50 unvaccinated workers in National Laboratory for public health, which is the Reference laboratory in Sudan.

1.4. Objectives

General objective:

To study the effect of handling of waste on liver functions among laboratory handlers.

Specific objectives:

- i. To assess the level of awareness of laboratory handlers.
- ii. To monitor the incidence of HBV and HCV among laboratory handlers.
- iii. To evaluate the levels of serum Bilirubin, total protein, albumin, alkaline phosphatase, Aspartate aminotransferase, and Alanine transaminase of laboratory handlers.

Chapter Two

2. Materials and Methods

2.1. Materials

2.1.1. Study design: This is cross sectional study.

2.1.2. Study area: The study was conducted in laboratory waste handler in National laboratory for public health.

2.1.3. Study population: This study included 50 unvaccinated waste handlers in National laboratory for public health since 2006 to March 2015.

Inclusion criteria: All laboratory waste handlers in National laboratory for public health since 2006 (who were examined for HBV, HCV) were included.

Exclusion criteria: All laboratory waste handlers in National laboratory for public health worked after 2006 were excluded.

2.1.4. Samples: About 5ml of venous blood were collected from each worker. The samples collected under aseptic conditions and placed in sterile plane containers, and after clotting centrifuged for 3 minutes at 3000 RPM to obtain serum, then the obtained serum were kept at -20c till the time of analysis.

2.1.5. Ethical consideration:

The study protocol was cleared by consent of the general manager and monitor of the National laboratory for public health.

Consent of the head department of virology and clinical chemistry lab.

Informed consent was obtained from all laboratory waste handlers.

2.1.6. Equipments:

- ELIZA, Hitachi (full automated).
- Centrifuge
- Sterile plane containers
- Disposable syringes
- 70% alcohol
- Tourniquets
- Cotton
- Micropipettes (automatic pipettes)
- Automatic pipettes 1ml.

2.1.7. Reagents:

Reagents of Hepatitis B virus:(Appendix II)

Reagents of Hepatitis C virus(AppendixIII):

Reagents of glutamate pyruvate tramsaminase (GPT) :(Appendix IV)

Reagents of glutamate oxaloacetate transaminase (GOT) (Appendix V)

Reagents of Alkaline phosphatase(ALP):(Appendix VI)

Reagents of Total protein : (Appendix VII)

Reagents of Albumin:(Appendix VIII)

Reagents of total bilirubin:(Appendix IX)

2.1.8. Data analysis: All data is arranged and analyzed for making some good recommendation on safe handling of laboratory waste, further more data analyzing is important to achieve the objective that were set earlier. Data was analyzed by using the SPSS computer program version 19, by using one sample T test program for analyzed LFT,(the mean is significant at $p \leq 0.05$) and frequency per percentage of list variables.

2.1.9. Data collection: Data were collected by using pre-tested and well structured check list; I used this method because it's best method for collection of correct data, due to different levels of education for those workers.

2.1.10. Quality control: To insure the quality of laboratory results, the standard Operating procedures of the company was followed every time and all tests were performed by experienced medical laboratory technologist. Furthermore known positive and negative control sera provided by the manufacture of the kits were used for every panel of the test.

2.2. Methodology

(1). Detection of Hepatitis B virus: (Appendix II)

Principle of method: The third generation enzyme immune assay is based on amicroplate well coated with monoclonal antibodies against a determinant of HBsAg. Patient serum is added to microwell s together with asecond mouse monoclonal antibody .conjugated with Hoeseradish peroxidase(HRP) and direct against epitope of the HBsAg . The specific immunocomplex, formed in the presence of HBsAg in the sample, is captured by the solid phase.

At the end of the incubation, microwells are washed to remove unbound serum protein and Hoeseradish peroxidase (HRP) conjugate.

The chromogen /substrate (TMB) is then add and, in the presence of captured HBsAg immunocomplex,the colored end-product after blocking the enzymatic reaction, its optical density is measured by ELISA reader. The color intensity is proportional to the amount of HBsAg present in the sample.

Quality control: A check is perform on the control /calibrator any time the kits used in order to verify whether the expected OD450nm or S/CO value have been matched in the analysis.

(2). Detection of Hepatitis C virus: (Appendix III)

Principle of method: Microplates are coated with HCV –specific antigen derived from (core peptide, recompenantNS3, NS4 and NS5 peptide) regions encoding for conservative and immunodominant antigenic determinant. The solid phase is first treated with the diluted sample and HCV Ab is captured, if present, by the antigen.

After washing out all the other component of the sample, in the second incubation bound HCV antibodies ,IgG and IgM as well, are detected by the addition of polyclonal specific anti IgG,IgM antibodies label with peroxides (HRP).

The enzyme captured on solid phase, acting on the substrate / chromogen mixture, generate an optical signal that is proportional to the amount of HCV present on the sample.

Quality control: A check is perform on the control /calibrator any time the kits used in order to verify whether the expected OD450nm or S/CO value have been matched in the analysis.

(3). Liver function test:

I. Estimation of glutamate pyruvate tramsaminase (ALT): (Appendix IV)

Principle of method: α -ketoglutarate + L-Alanine $\xrightarrow{\text{GPT}}$ L-glutamate + pyruvate

The increase in pyruvate determined by indicator reaction catalyzed by LDH.

Pyruvate + NADH + H⁺ $\xrightarrow{\text{LDH}}$ L-Lactate + NAD⁺

NADH is oxidized to NAD. The rate of NADH decreased directly proportional to the rate of formation of pyruvate and GPT activity.

II. Estimation of glutamate oxaloacetate transaminase (AST): (Appendix V)

Principle of method: α -ketoglutarate + L-Aspartate $\xrightarrow{\text{GOT}}$ L-glutamate + oxaloacetate

The increase in oxaloacetate determined by indicator reaction catalyzed by NAD⁺.

oxaloacetate + NADH + H⁺ $\xrightarrow{\text{MDH}}$ L-malate + NAD⁺

NADH is oxidized to NAD. The rate of NADH decreased directly proportional to the rate of formation of oxaloacetate and GOT activity.

III. Estimation of Alkaline phosphatase: (Appendix VI)

Principle of method:

P-Nitrophenylphosphate + H₂O $\xrightarrow{\text{AP}}$ p-Nitrophenate + phosphate

P-Nitrophenol is present as yellow coloured p-Nitrophenate. The increase of absorbance per min at 405nm is proportional to the enzyme activity.

IV. Estimation of Total protein: (Appendix VII)

Principle of method: Colorimetric determination of total protein based on the principle of the biuret reaction, in alkaline solution divalent copper react with protein peptide bond to blue colored complex.

Potassium iodide prevents autoreduction of copper and sodium potassium tartrate prevents the precipitation of copper hydroxide. The intensity of colour is proportional to concentration of protein.

V. Estimation of Albumin: (Appendix VIII)

Principle of method: At PH value of 4.1 albumins displays asufficiency cationic character to be able to bind with Bromocresol green (BCG), an anionic dyestuff, to form a blue –green complex. The intensity of colour is proportional to concentration of albumin.

VI. Estimation of total bilirubin: (Appendix IX)

Principle of method: Total bilirubin reacts in presence of DMSO or caffeine with diazotized sulfanic acid to form ared azo dye.

Chapter Three

3. Results

Fifty non vaccinated workers in national laboratory for public health where who have been tested and found negative for HBsAg and HCV since 2006 and enrolled in this study, to study the effect of handling of waste on their liver functions. After collection of required data and suitable samples for tests, the tests were done and the results were analyzed by using SPSS computer program and the results were as follow:

An adequate awareness about HBV and HCV infection over all workers were 48 % (Figure 3.1), During work only 62% were wearing a protective cloth (Figure 3.2) and 84 % were wearing gloves while handling laboratory waste (Figure 3.3), Just about 24 % had segregate the waste (Figure 3.4), 18 % using sharp container while handling sharpener (Figure 3.5) and Only 8% had got pricked accidentally by handle needle (Figure 3.6).

When measuring HBsAg and HCV by (ELISA) we found that only one worker (2 % of total) had infected with HBV during last ten years (Figure 3.7) and no one had infected with HCV.

Liver Function Tests including: total protein, albumin, total bilirubin, ALP, AST and ALT were done for those workers, and one sample T test was used to compare the result mean with the mean value.

Table 3.1: Shows a significant decrease in total bilirubin, total protein, albumin, alkaline phosphates, aspartate aminotransferase, P. value prospectively (0.007, 0.000, 0.0003, 0.000, and 0.026) and insignificant decrease for Alanine aminotransferas, P. Value 0.435.

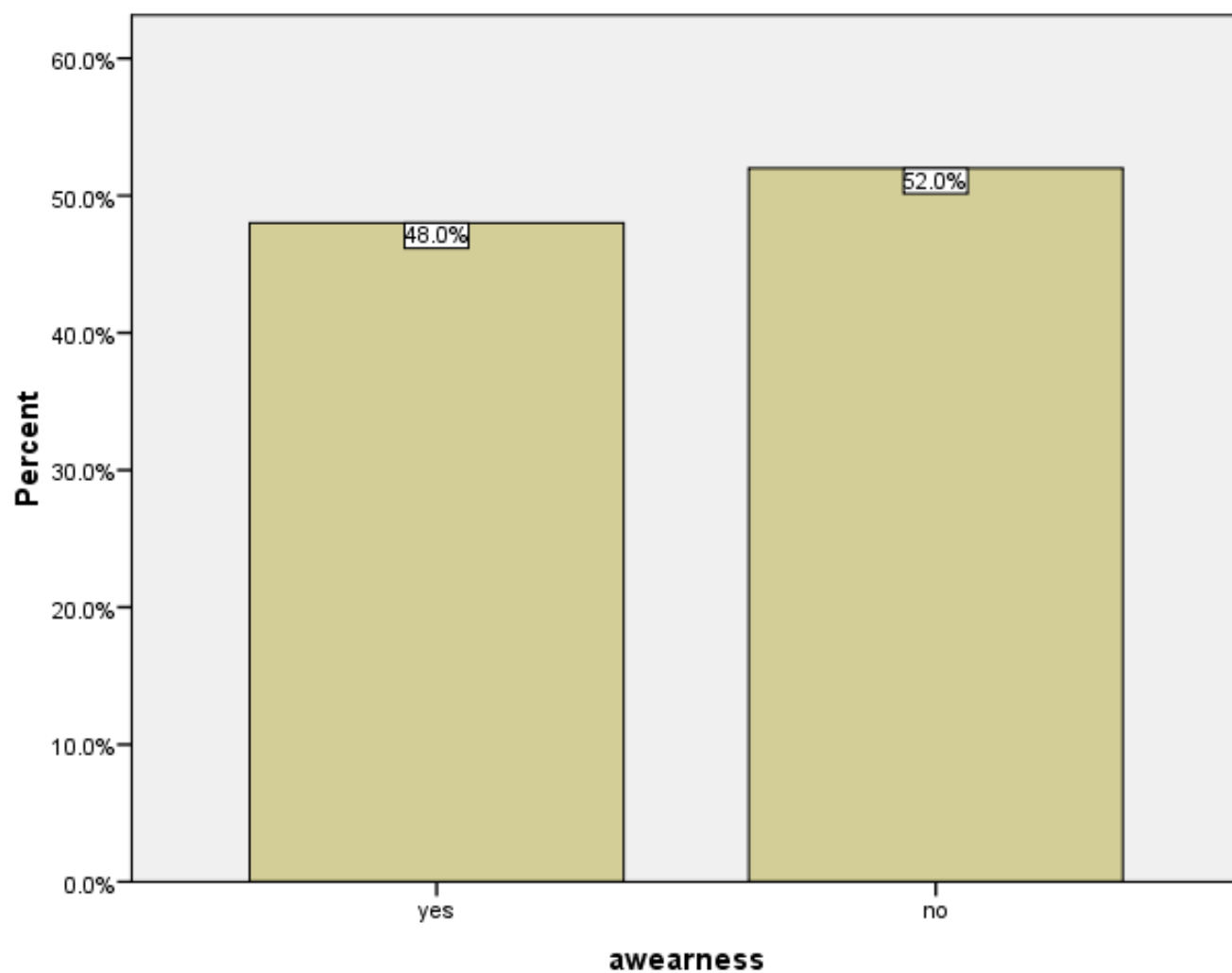


Figure3.1:
Awearness about HBV and HCV infection

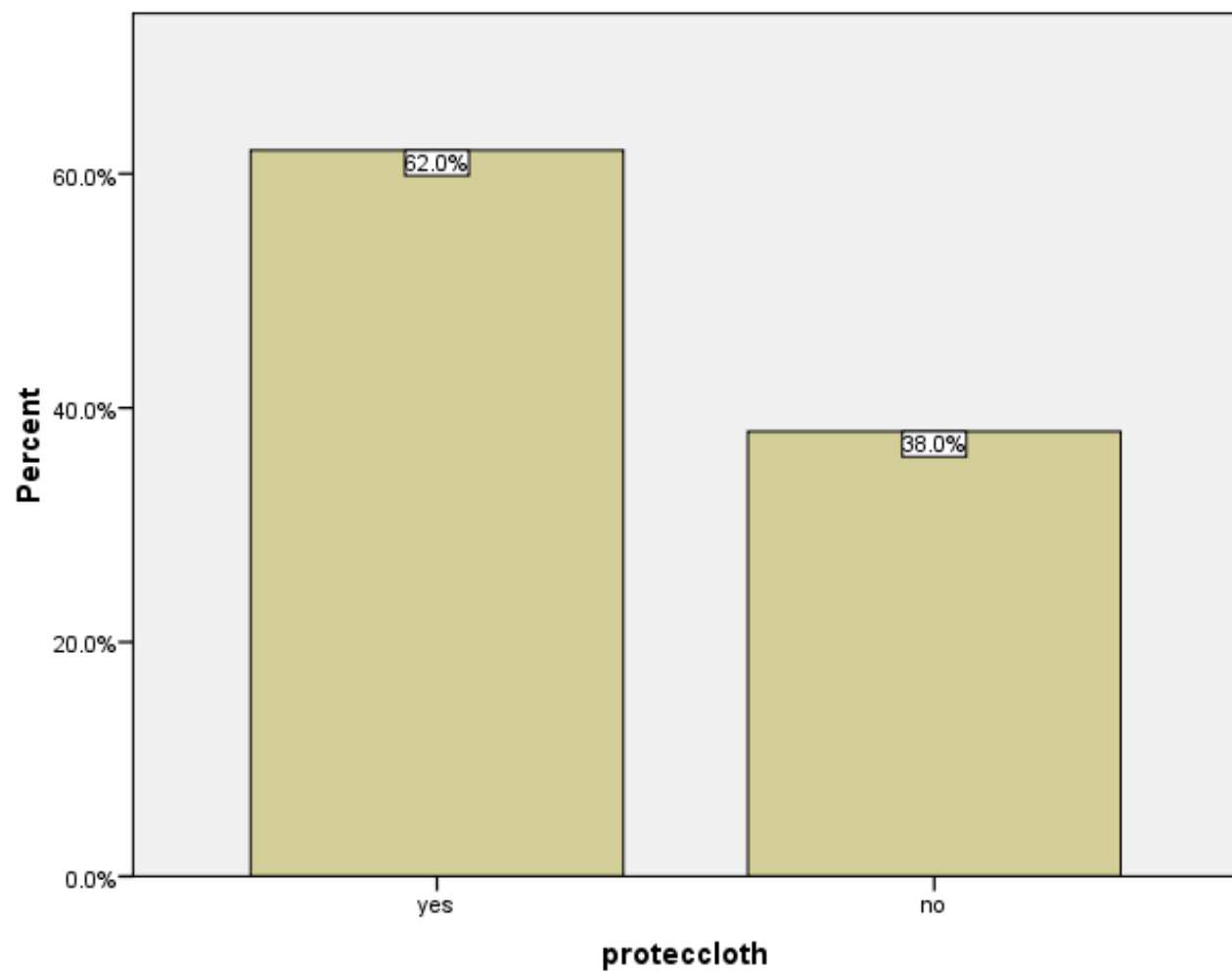
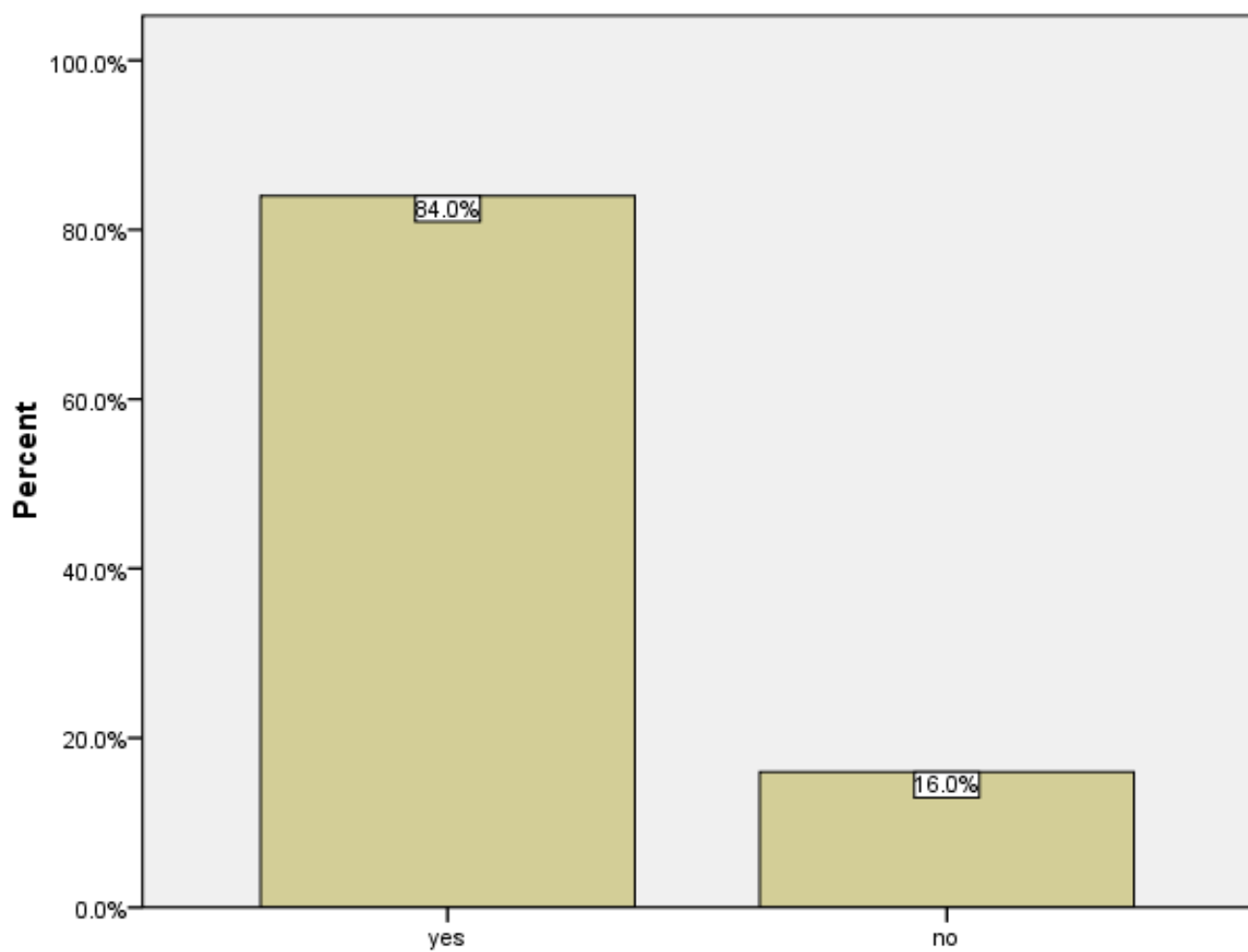


Figure 3.2:
wearing a protective cloth



gloves
Figure 3.3:
wearing gloves

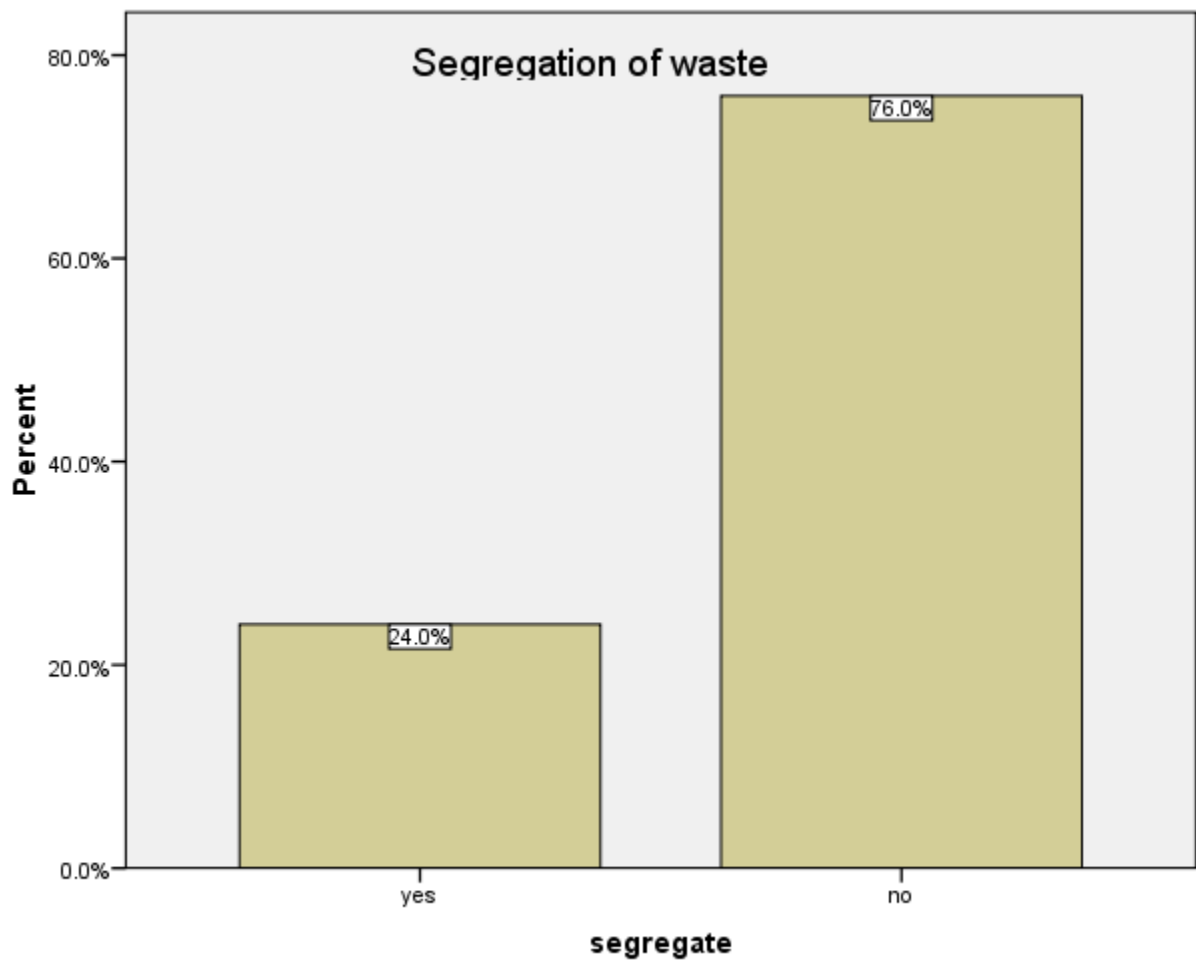


Figure 3.4: Segregation of waste

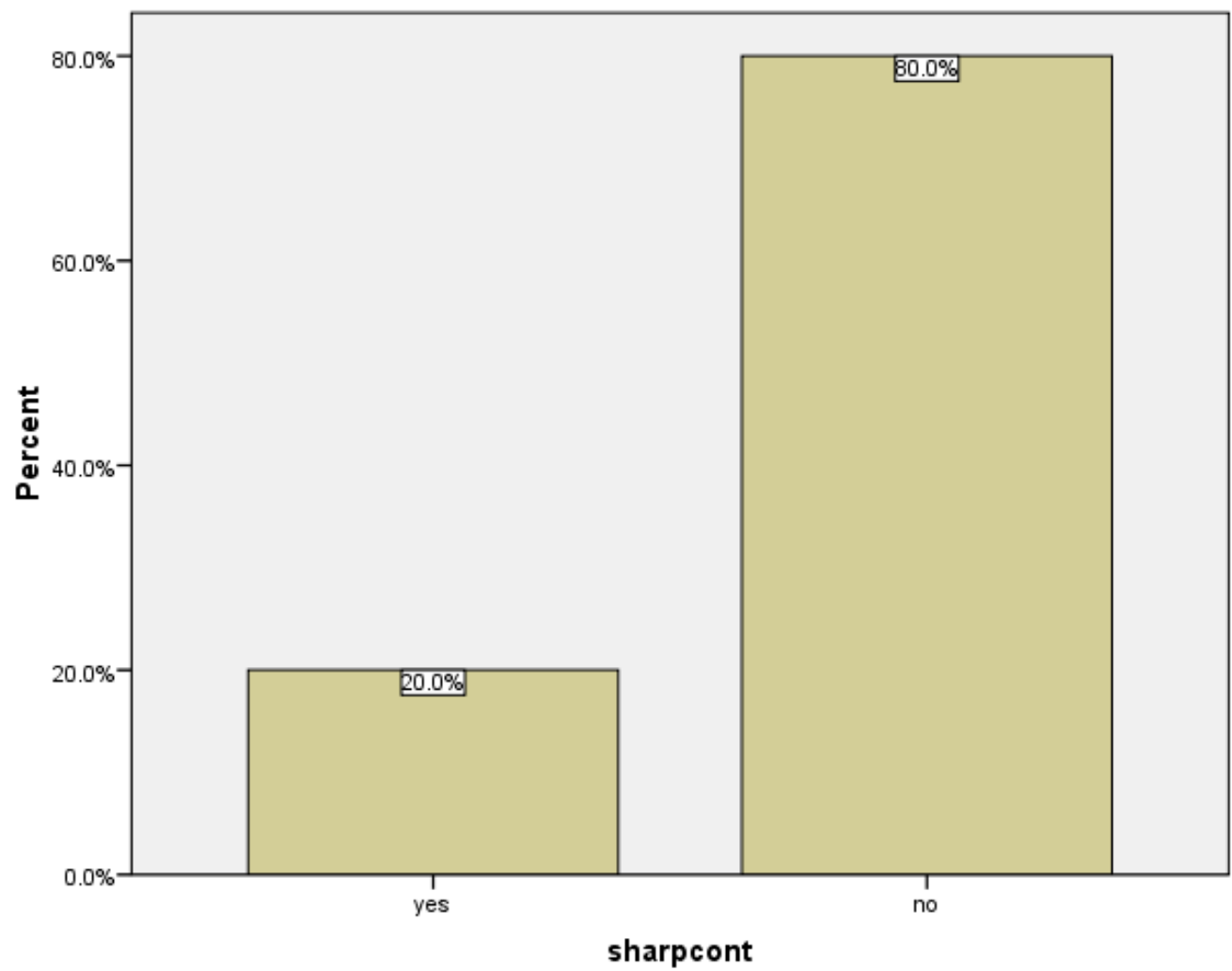


Figure 3.5 Using sharp container while handling sharpener

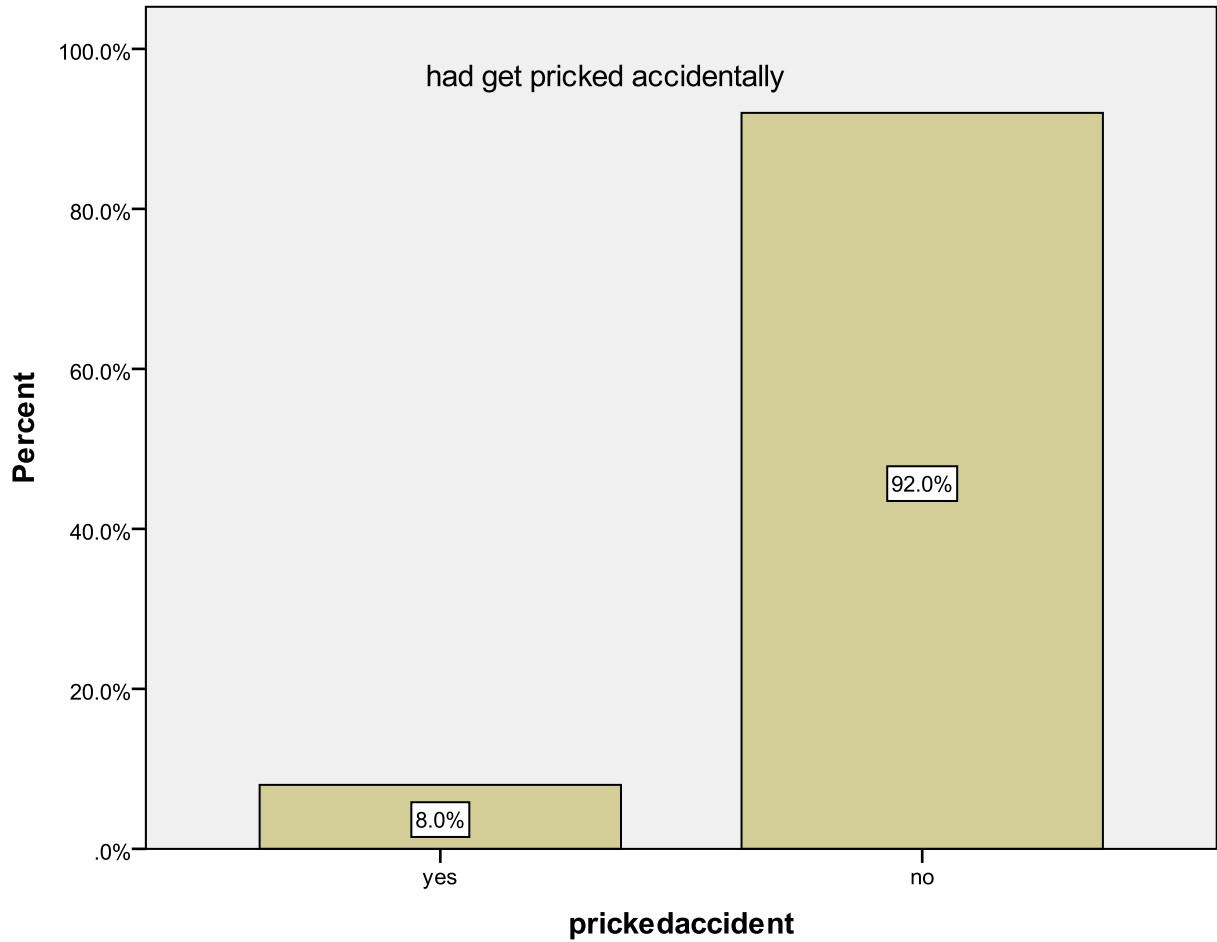


Figure 3.6. Had got pricked accidentally by handle needle.

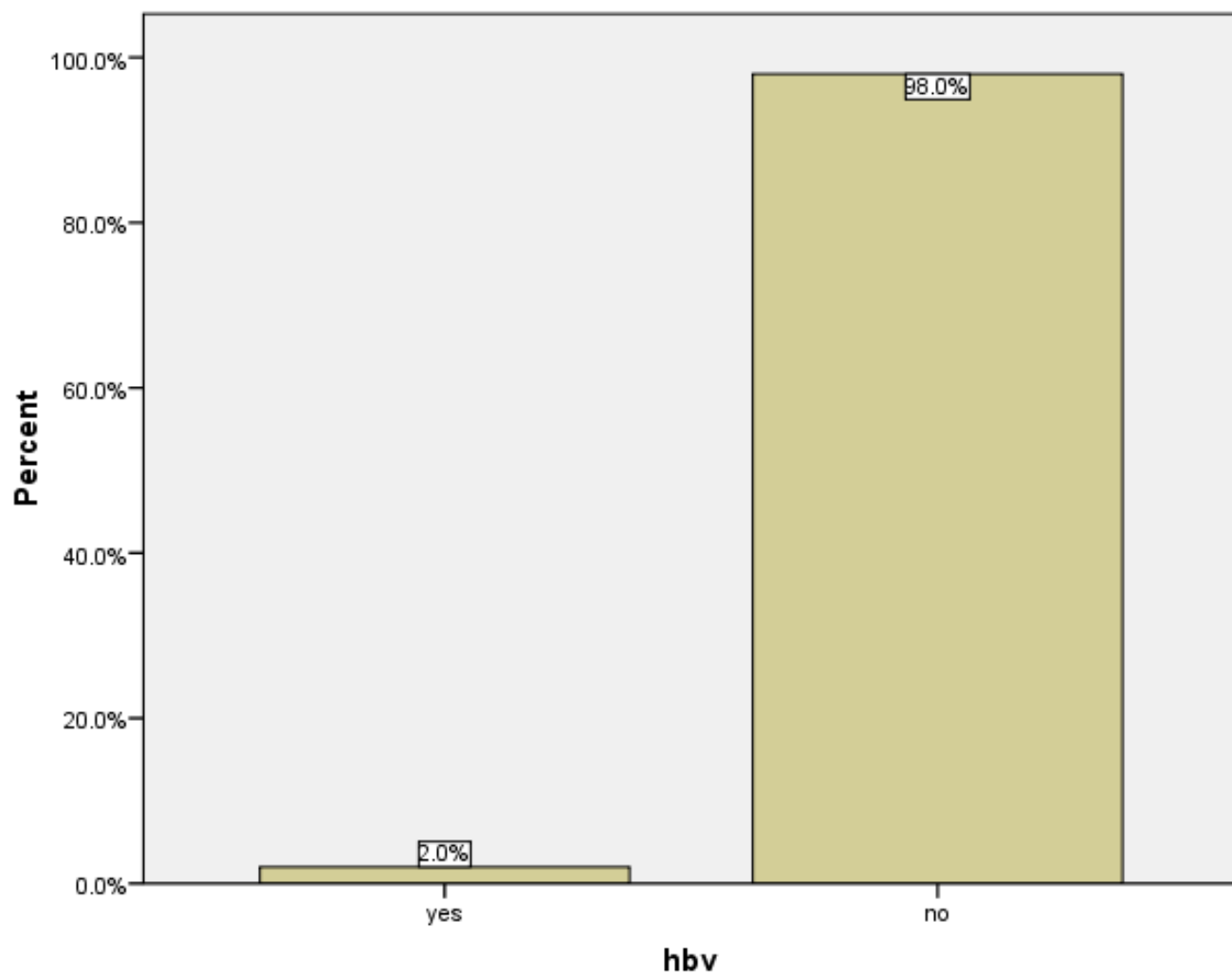


Figure 3.7: HBV +ve

Table 3.1: The result of liver function tests of the workers.

Variable	Normal Mean value	Mean±SD	P.value
S/ Total Protein (g/dl)	7.4	6.090 06028	0.000
S/ Albumin (g/dl)	4.3	4.676 0.8551	0.003
S/ ALP (U/l)	220	144.98 58.293	0.000
S/ AST (U/l)	30	24.20 12.943	0.000
S/ ALT (U/l)	20	18.54 13.114	0.435
S/Total bilirubin (mg/dl)	0.5	0.874 0.9417	0.007

One sample T test, P. value < 0.05 was considered statistical significance.

Chapter Four

4. Discussion, conclusion and Recommendations

4.1. Discussion

Laboratory waste handlers are at greater risk at contacting blood borne disease due to their constant contact with infectious waste or sharps contaminated with blood ([Shankargouda Patil](#); et al(2013). No data about the prevalence of HBV or effect on liver function test among laboratory waste handlers is available in Sudan.

In the present study we found that the group of workers (50 individuals) who enrolled in the study haven't incidence with HBV or HCV in 2006.

As the results of this study, during this period, only one of them had infected with HBV and no incidence with HCV. Although none of them was immunized against hepatitis B virus, and there was no adequate awareness among the group. This evident when compared with data obtained in Ethiopia at Gondar town in University of Gondar Teaching Hospital, Northwest Ethiopia (2011) about HBV and HCV among medical waste handlers HBV was detected in 6 (6.0%) and HCV in 1 (1.0%). Also similar study found in Tripoli, Libya with reported rate of 2.3% HBV; whereas 2.7% HCV positivity which was slightly higher ([Belay](#). et al, 2012). Lower and higher prevalence rates were also detected as compared to similar study populations in different parts of the world.

For liver function tests the statistical analysis showed a significant decrease in serum levels of total bilirubin, total protein, albumin, alkaline phosphates and aspartate aminotransferase with P.value prospectively (0.007, 0.000, 0.0003, 0.000, and 0.026) and insignificant decrease for Alanine aminotransferas with P. value 0.435. when comparing the results with similar study conduct in solid waste disposal workers in Port Harocurt, Nigeria, the AST values increased significantly for solid waste workers with a mean AST concentration of $(11.19 \pm 2.36 \mu\text{L}^{-1})$ and $8.97 \pm 4.07 \mu\text{L}^{-1}$ for the control subjects, and mean total bilirubin increased

progressively as the number of years of exposure increased (19.00 $\mu\text{mol L}^{-1}$). The peak value was for workers exposed for about 7 year. Other liver function tests values did not show much difference ([Wachukwu](#) . et al, 2007).

4.2. Conclusion

1. Workers who enrolled in the study, shows a significant impairment in serum levels of total bilirubin, total protein, albumin, alkaline phosphates, aspartate aminotransferase, and insignificant impairment for Alanine aminotransferas.
2. Manual collection of medical wastes increased the observed accidents.

4.3. Recommendations

1. Awareness, precaution and protection program should be regularly done in order to prevent HBV and HCV.
2. Strict implementation of biomedical waste management with planned and clear policies for segregation of waste, handle shaping in safety boxes, and implementation of vaccine should be established.
3. Laboratory workers should be trained and periodically monitored for laboratory waste disposal.
4. It should be insured that the injuries happened to the workers are reported to the safety officer.

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Appendix I

Questionnaire

- Name:

- Duration of work:

- Age:

- Gender: male () Female ()

- Wearing of gloves: Yes () NO ()

- Wearing of protective cloth: Yes () NO ()

- Segregation of waste: Yes () NO ()

- Education level: Primary () Secondary () University ()

- Knowledge of deal with waste: Yes () NO ()

- Training: Yes () NO ()

- Handle the sharpening in a safety box. Yes () NO ()

- Frequency of supervisor visit :None () Rare () Always ()

- Accident: Yes () NO ()

- Vaccination: Yes () NO ()

Appendix II



HBs Ag One Step
Enzyme Immunoassay Test
Product N°:4210 (96 test)
Product N°:4211 (192 test)

▽ 96-192 Test

[IVD]

SUMMARY OF PROCEDURE

1. Pre-wash the plate once (350 µl).
2. **STEP 1** Place 150 µl of Negative Control, Calibrator, Positive Control and serum (-) samples in the wells of the strips, leaving one well for the blank. Then add 100 µl of diluted Enzyme Conjugate.
3. Incubate for 120 min. at 37°C
4. Wash 4-5 times (350 µl)
5. **STEP 2** Place 200 µl of Chromogen/Substrate in each well
6. Incubate for 30 min. at room temperature (18-30°C)
7. **STEP 3** Add 100 µl of Stop Solution
8. Read absorbance at 450 nm & 620 nm.

INTENDED USE

Third generation Enzyme Immunoassay (ELISA) for the one-step qualitative determination of Hepatitis B surface Antigen in human (-) sera. The kit may be used for the screening of blood units and the follow-up of HBV-infected patients.
 For *in vitro* diagnostic use only.

SUMMARY AND EXPLANATION

The Hepatitis B Virus (HBV) is a human pathogen DNA virus with a world-wide distribution among geographic areas and population groups. He is one of the five strictly hepatotropic viruses, together with the A, C, Delta and E viruses. All of these viruses can cause acute disease, with symptoms that ranged between mild asymptomatic infection to severe fulminant hepatitis¹⁻³. The typical symptoms are yellowing of the skin and eyes (jaundice), dark urine and extreme fatigue, nausea, vomiting and abdominal pain⁴. HBV can cause chronic infection when the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer⁴, particularly if the infection was acquired in childhood than as an adult^{5,6}. In this case the infection persists for more than six months. More than 90% of the infected adults will have an acute self-limiting infection¹. Young children are the most likely to develop chronic hepatitis infection. 90% of the infants affected by HBV infection in the first year and 30-50% of the infants affected into the first 4 years can develop chronic hepatitis, and 25% of these patients will death for liver cancer or cirrhosis⁴. HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Since 1991, WHO suggested to consider the hepatitis B vaccine into their national immunization programs. The therapy is mainly based on alfa-interferon to relieve symptoms^{5,6}. The response to this treatment is between 40 and 50% of patients with chronic active HBV infection^{5,6}. In the chronic HBV carriers there is no evidence of hepatic damage^{1,2}, the infection persists and the patient maintains the ability to transmit the virus. The transmission of the disease is mainly due to a parenteral contact (blood or blood products exchange, sexual contacts, perinatal spread from mother to newborn at the birth, unsafe injections and transfusions)⁴. The virus is not spread by contaminated food or water⁴. High prevalence of HBV infections are found in southern areas of Eastern and Central Europe. Lower prevalence (5%) is detected in Middle and Far East, and less than 1% in Western Europe and North America⁴.

In the HBV infection three phases can be identify: incubation, acute and coalescent, on the basis of several serological markers results. The first marker to appear in the serum is the hepatitis B surface antigen (HBsAg), detectable after 4-12 weeks after the infection and before the onset of the symptoms^{1,2,5}.

HBsAg is the most important protein in the external envelope of the virus, responsible of the acute and chronic viral infection⁷. This antigen contains the proteic determinat "a", common to all the virus subtypes, that can be immunologically distinguish in two subgroup: "ay" and "ad".

PRINCIPLE OF THE PROCEDURE

The third generation "HBsAg One Step" enzyme immunoassay is based on a microtiterplate wells coated with mouse monoclonal antibodies against "a" determinat of HBsAg. Patient's serum (-) is added to the microwells together with a second mouse monoclonal antibody, conjugated with Horseradish Peroxidase (HRP) and directed against a different epitope of the HBsAg. The specific immunocomplex, formed in the presence of HBsAg in the sample, is captured by the solid phase.

At the end of the one-step incubation, microwells are washed to remove unbound serum proteins and HRP conjugate.

The chromogen/substrate (TMB) is then added and, in the presence of captured HBsAg immunocomplex, the colorless substrate is hydrolyzed by the bound HRP conjugate to a colored end-product. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.

The color intensity is proportional to the amount of HBsAg present in the sample.

REAGENTS

Code	4210	4211
Test N°	96	192
Microplate	1	2
Negative Control	2x1.8 ml	3x1.8 ml
Positive Control	1x1.8 ml	2x1.8 ml
Calibrator	1 lyo	2 lyo
Wash Buffer 20x concentrate	1x60 ml	2x60 ml
Enzyme Conjugate 20x concentrate	1x 0.8 ml	1x1.6 ml
Enzyme Conjugate Diluent	1x16 ml	1x32 ml
Chromogen Substrate	2x20 ml	2x40 ml
Stop Solution	1x20 ml	1x40 ml

Bring to room temperature before use

Antibody Coated Wells (Microplate) Microplate with breakable wells coated with affinity purified mouse monoclonal antibodies against "a" determinant of HBsAg (anti-HBsAg) and sealed into a bag with desiccant.

Negative Control Ready to use. Goat serum with 0.09% Na-azide and 0.1% Kathon GC as preservatives. The negative control is pale yellow color coded.

Positive Control Ready to use. 2%BSA with non infectious recombinant HBsAg into 100 mM Tris HCL buffer pH 7.4+/-0.1, and 0.1% Kathon GC as preservatives. The positive control is green color coded.

Calibrator Lyophilized reagent. To be reconstitute with EIA grade water as reported in the label. Contains 4% bovine serum proteins, non infectious recombinant HBsAg at 0.5 IU/ml (2nd WHO

International Standard for HBsAg, NIBSC code 00/588), 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

Note: The volume necessary to reconstitute the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

Wash buffer concentrate 20X

20X concentrated solution. Once diluted, the working solution contains 10 mM phosphate buffer pH 7.0 \pm 0.2, 0.05% Tween 20 and 0.1% Kathon GC.

Enzyme Conjugate Diluent

Ready to use. 10% Fetal bovine serum, 1% Purified mouse monoclonal antibody 10 mM Trisplex buffer pH 7.6 \pm 0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

Enzyme Conjugate 20X concentrated

Concentrated (20X) Horseradish Peroxidase (HRP) labeled mouse monoclonal antibody to HBsAg, determinant "a", MOPS buffer pH 6.2 - 6.7. Contains bovine protein preserved with 0.02% methylisothiazolone and bromonitrodioxane, 20 ppm Proclin 300.

Chromogen Substrate

Ready to use 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

Stop Solution

Ready to use 0.3 M H₂SO₄ solution.

Adhesive sealing foils

OTHER MATERIALS REQUIRED

Calibrated Micropipettes (150 μ l, 100 μ l and 50 μ l) and disposable plastic tips.
EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
Timer with 60 minute range or higher.
Absorbent paper tissues.
Calibrated ELISA microplate thermostatic incubator (dry or wet), capable to provide shaking at 1300 rpm \pm 150, set at +37°C.
Calibrated ELISA microwell reader with 450nm (reading) and possibly with 620-630nm (blanking) filters.
Calibrated ELISA microplate washer.
Vortex or similar mixing tools.

STORAGE AND STABILITY OF REAGENTS

Reagents must be stored at 2/8°C.
The expiry date is printed on each component and on the box label.
Reagents have a limited stability after opening and/or preparation.

Microplate Use: open the package on the opposite end from the code which is useful for identification purposes, remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the dessiccant, expell the air and seal by pressing the closure.

Negative Control Ready to use. Mix well on vortex before use.

Positive Control Ready to use. Mix well on vortex before use.

Calibrator Add the volume of EIA grade water reported on the label to the lyophilized powder. Let fully reconstitute and gently mix on vortex.
Note: The solution is not stable. Store the Calibrator frozen in aliquots at -20°C.

Wash buffer concentrate The whole content of the concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be

present into the vial, take care to dissolve all the content when preparing the solution. In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.

Note: Once diluted, the Wash solution is stable for 1 week at +2-8° C.

Enzyme Conjugate The working solution is prepared by diluting the concentrated Enzyme Conjugate with the Enzyme Conjugate Diluent (e.g. 100 μ l Conjugate + 1.9 ml Diluent). Mix well on vortex before use. The working solution is not stable. Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

Important note: Prepare only the volume necessary for the work of the day as the diluted conjugate is not stable.

Chromogen Substrate Ready to use. Mix well on vortex before use. Avoid contamination of the liquid with oxidizing chemicals, airdriven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces. If this components has to be transferred use only plastic and, if possible, sterile disposable container.

Stop Solution Ready to use. Mix well on vortex before use.

WARNINGS AND PRECAUTIONS

For in vitro Diagnostic Use Only.

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between serum/(-) samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and

from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..

15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

17. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm 2\%$.

18. The ELISA incubator has to be set at +37°C (tolerance of $\pm 1^\circ\text{C}$) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

19. In case of shaking during incubations, the instrument has to ensure 350 rpm ± 150 . Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.

20. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 μl /well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the section "QUALITY CONTROL". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

21. Incubation times have a tolerance of $\pm 5\%$.

22. The microplate reader has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630 nm) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 2.0 ; repeatability $\geq 1\%$. Blanking is carried out on the well identified in the section "ASSAY PROCEDURE". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.

23. When using ELISA automated workstations, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "QUALITY CONTROL". The assay protocol has to be installed in the operating system of the unit and validated by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing.

The carry over effect must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.

24. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2-8°C, firmly capped.

SPECIMEN COLLECTION

1. Blood is drawn aseptically by venepuncture and (-) serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.

3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.

4. Haemolysed (red) and lipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as well as they could give rise to false positive results.

5. Sera and (-) can be stored at +2°-8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen sample should not be frozen/thawed more than once as this may generate particles that could affect the test result. If some turbidity is present or presence of microparticles is suspected after thawing, filter the sample on a disposable 0.2-0.8 μl filter to clean it up for testing.

PROCEDURE

PREASSAY PROCEDURE

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dilute the 20X concentrated Enzyme Conjugate with its Diluent as reported.
5. Reconstitute the Calibrator as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Automated assay

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense first 150 μl controls & calibrator, then all the samples and finally 100 μl diluted Enzyme Conjugate.

For the pre-washing step (point 1 of the Assay Procedure) and all the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the

instrument and taken into consideration by delaying the first washing operation accordingly.

Manual Assay

1. Place the required number of strips in the plastic holder and wash them once to hydrate wells. Carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 150 µl of the Negative Control in triplicate, 150 µl of the Calibrator in duplicate and then 150 µl of the Positive Control in single followed by 150 µl of each of the samples.
4. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620 nm. (samples show OD values higher than 0.100).
5. Dispense 100 µl diluted Enzymatic Conjugate in all wells, except for A1, used for blanking operations.
6. Following addition of the conjugate, check that the color of the samples have changed from yellowish to red and then incubate the microplate for 120 min at +37°C.
7. When the first incubation is over, wash the microwells as previously described (section "WARNINGS AND PRECAUTION")
8. Pipette 200 µl Chromogen/Substrate into all the wells, A1 included.
9. Incubate the microplate protected from light for 30 min at room temperature. Wells dispensed with the positive control, the calibrator and positive samples will turn from clear to blue.
10. Pipette 100 µl Stop Solution into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 8. Addition of the acid solution will turn the positive control, the calibrator and positive samples from blue to yellow.
11. Measure the color intensity of the solution in each well, as described in section "WARNINGS AND PRECAUTIONS", using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1.

		Microplate											
		1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2											
B	NC	S3											
C	NC	S4											
D	NC	S5											
E	CAL	S6											
F	CAL	S7											
G	PC	S8											
H	S1	S9											

Legenda: BLK = Blank; NC = Negative Control; CAL = Calibrator; PC = Positive Control; S = Sample

IMPORTANT NOTES

1. Pre washing is fundamental to obtain reliable and specific results both in the manual and in the automatic procedures. Do not omit it!
2. Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.
3. Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.
4. If the procedure is carried out on shaking, be sure to deliver the rpm reported for in section "WARNINGS AND PRECAUTIONS" as otherwise intra-well contamination could occur.
5. Do not expose to strong direct light as a high background might be generated.
6. If the second filter is not available, ensure that no fingerprints or dust are present on the external bottom of the microwell before reading at 450nm. They could generate false positive results on reading.
7. Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.
8. When samples to be tested are not surely clean or have been stored frozen, filter the sample on a disposable 0.2-0.8 µm as long

as it is far less sensitive to interferences due to hemolysis, hyperlipaemia, bacterial contamination and fibrin microparticles.

QUALITY CONTROL

A check is performed on the Controls/Calibrator any time the kit is used in order to verify whether the expected OD_{450nm} or S/Co values have been matched in the analysis.

Ensure that the following results are met:

Blank well (Bw):	OD _{BW} < 0.100;
Negative Control (NC):	OD _{NC} value after blanking < 0.050;
Calibrator 0.5 IU/ml (2 nd WHO Std):	S/CO ≥ 2;
Positive Control (PC):	OD _{PC} > 1.000

If the results of the test match the requirements stated above, proceed to the next section.

TROUBLESHOOTING

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well: OD _{450nm} > 0.100	1. that the Chromogen/Substrate solution has not become contaminated during the assay.
Negative Control: OD _{450nm} after blanking > 0.050	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator: S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of Negative Control instead of Calibrator) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control: OD _{450nm} < 1.000	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control. In this case, the negative control will have an OD _{450nm} value > 0.050). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

RESULTS

The test results are calculated by means of a cut-off value determined on the mean OD_{450nm} value of the Negative Control (NC) with the following formula:

$$\text{Cut-Off (Co)} = \text{NC} + 0.050$$

The value found for the test is used for the interpretation of results as described in the next paragraph.

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD_{450nm} (S) and the Cut-Off value (Co), mathematically S/Co, according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient is not infected by HBV and that the blood unit may be transfused.

Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample; the blood unit should not be transfused.

A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.
2. Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0.8 µ filter to remove any microparticles interference. Then, if still positive, the sample has to be submitted to a confirmation test before a diagnosis of viral hepatitis is released.
3. When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.
4. Diagnosis of viral hepatitis infection has to be taken and released to the patient by a suitably qualified medical doctor.

An example of calculation is reported below.

The following data must not be used instead of real figures obtained by the user.

Negative Control: 0.012 – 0.008 – 0.010 OD_{450nm}
Mean Value: 0.010 OD_{450nm}
Lower than 0.050: Accepted

Positive Control: 2.489 OD_{450nm}
Higher than 1.000: Accepted

Cut-Off: 0.010+0.050 = 0.060

Calibrator: 0.350 – 0.370 OD_{450nm}
Mean value: 0.360 OD_{450nm}; S/Co = 6.0
S/Co higher than 2.0: Accepted

Sample 1: 0.028 OD_{450nm}
Sample 2: 1.690 OD_{450nm}
Sample 1 S/Co: < 0.9 = negative
Sample 2 S/Co: > 1.1 = positive

PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

Limit of detection

The limit of detection of the assay has been calculated by means of the following international preparations:

1. standards for HBsAg - ay and ad subtypes - supplied by Paul Erlich Institute (from preparation 50.000 U/ml and 100 U/ml respectively);
2. NIBSC 2nd British working standard (French/UK standard code 99/640-005-WI)
3. 1st WHO international standard, code n° 80/549.
4. 2nd WHO international standard, NIBSC code 00/588.

Results of Quality Control are given in the following table:

Standard Preparation		
PEI		
"ad" subtype	0.0125	PEI U /ml
"ay" subtype	0.0125	PEI U /ml
NIBSC	0.03	ng/ml
WHO 1 st standard	0.05	IU/ml
WHO 2 nd standard	0.1-0.05	IU/ml

The panel # 806, supplied by Boston Biomedical Inc., USA, was also tested to define the limit of sensitivity.

Results are as follows :

BBI Panel 806

Member ID	HBsAg Subtype	BBI ng/ml	Abbott ng/ml	PEI IU/ml	Lot 1 S/Co	Lot 2 S/Co	Lot 3 S/Co
806-1	ad	2,5	3,1	0,31	9,5	9,4	9,7
806-2	ad	1,5	1,6	0,17	5,1	5,0	5,3
806-3	ad	1	1	0,1	3,2	3,2	3,3
806-4	ad	0,8	0,9	0,09	2,6	2,4	2,8
806-5	ad	0,6	0,7	0,07	2,3	2,1	2,4
806-6	ad	0,5	0,5	0,06	2,0	1,8	1,9
806-7	ad	0,4	0,5	0,05	1,7	1,6	1,8
806-8	ad	0,3	0,3	0,03	1,4	1,3	1,5
806-9	ad	0,2	0,2	0,02	1,2	1,1	1,2
806-10	ad	0,1	0,1	0,01	0,8	0,7	0,8
806-11	ay	2,4	1,8	0,21	10,5	10,7	10,6
806-12	ay	1,3	1	0,11	5,3	5,6	5,5
806-13	ay	0,9	0,6	0,07	4,7	4,9	4,9
806-14	ay	0,8	0,5	0,06	3,9	4,0	4,0
806-15	ay	0,6	0,4	0,05	2,6	2,7	2,5
806-16	ay	0,5	0,4	0,04	2,4	2,5	2,5
806-17	ay	0,4	0,3	0,03	1,7	1,8	1,9
806-18	ay	0,3	0,3	0,03	1,6	1,7	1,7
806-19	ay	0,2	0,2	0,02	1,4	1,6	1,5
806-20	ay	0,1	0,1	0,01	1,1	1,2	1,1
806-21	negative	0	0	0	0,2	0,2	0,2

In addition two panels of sensitivity supplied by EFS, France, have been tested and have given the following results:

Sensitivity panel EFS, France

Echantillon	(c) théorique d'Ag HBs ng/ml	Mean OD _{450nm}	S/Co	Ortho S/Co
HB 1	0.10	0.039	0.7	0.6
HB 2	0.20	0.082	1.4	1.2
HB 3	0.30	0.116	2.0	1.8

HB 4	0.45	0.161	2.7	2.8
HB 5	0.66	0.210	3.6	3.9
HB 6	diluent	0.009	0.2	0.2

Sensitivity panel SFTS, France, Ag HBs 2004

Sample ID	Characteristics	ng/ml	S/Co
151	Adw2 + ayw3	2.24 ± 0.15	10.8
152	Adw2 + ayw3	1.15 ± 0.06	5.8
153	Adw2 + ayw3	0.95 ± 0.05	5.0
154	Adw2 + ayw3	0.58 ± 0.04	3.3
155	Adw2 + ayw3	0.50 ± 0.03	2.6
156	Adw2 + ayw3	0.36 ± 0.02	1.9
157	Adw2 + ayw3	0.23 ± 0.02	1.3
158	Adw2 + ayw3	0.13 ± 0.02	0.7
159	Adw2 + ayw3	0.06 ± 0.01	0.4
160	Adw2 + ayw3	0.04 ± 0.01	0.2
161	Adw2	0.5 – 1.0	2.8
162	Adw4	0.5 – 1.0	2.6
163	Adr	0.5 – 1.0	3.0
164	Ayw1	0.5 – 1.0	2.9
165	Ayw2	0.5 – 1.0	3.9
166	Ayw3	0.5 – 1.0	4.6
167	Ayw3	0.5 – 1.0	4.3
168	Ayw4	0.5 – 1.0	4.2
169	Ayr	0.5 – 1.0	3.6
170	diluent	/	0.1

Diagnostic sensitivity

The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.

Positive samples were collected from different geographical regions (including therefore both ad and ay subtypes) and from different HBV pathologies (acute, a-symptomatic and chronic hepatitis B).

An overall value of 100% has been found in the study conducted on a total number of more than 400 samples.

A total of more than 20 seroconversion and performance panels have also been studied. Results obtained by examining two Panels supplied by Boston Biomedica Inc., USA, are reported below.

BBI PHM 916

Sample ID	HBsAg conc. ng/ml	Delta B. EIA S/Co	Ortho EIA S/Co	Sorin EIA S/Co
01	< 0.1	0.4	0.2	0.0
02	< 0.1	0.3	0.2	0.1
03	< 0.1	0.3	0.2	0.0
04	< 0.1	0.4	0.1	0.0
05	< 0.1	0.4	0.2	0.0
06	< 0.1	0.4	0.6	0.1
07	< 0.1	0.4	0.2	0.1
08	< 0.1	0.5	0.2	0.1
09	0.2	0.9	0.4	0.2
10	0.5	1.4	2.2	1.2
11	2.3	4.5	10.1	6.2

BBI PHM 923

Sample ID	HBsAg conc. ng/ml	Delta B. EIA S/Co	Ortho EIA S/Co	Sorin EIA S/Co
01	< 0.1	0.4	0.1	0.0
02	< 0.1	0.4	0.2	0.1
03	< 0.2	1.2	1.4	0.9
04	< 0.5	1.4	2.6	1.8

Diagnostic specificity

The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a kit registered in Italy by the Ministry of Health.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.

No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.

All the HBsAg known subtypes, "ay" and "ad", and isoforms "w" and "r", supplied by CNTS, France, were tested in the assay and determined positive by the kit as expected.

Samples derived from patients with different viral (HCV, HAV) and non viral pathologies of the liver that may interfere with the test were examined.

No cross reaction were observed.

The Performance Evaluation study conducted in a qualified external reference center on more than 5000 samples has provided a value of > 99.5%.

Precision

It has been calculated on three samples examined in replicates in different runs. The mean values obtained from a study conducted on two samples of different HBsAg reactivity, examined in 16 replicates in three separate runs (Standard Procedure) for three lots is reported below:

Lot # 1

Negative Sample (N = 16)

Mean values	1 st run	2 nd run	3 rd run	Average
OD _{450nm}	0.036	0.036	0.032	0.034
Std. Deviation	0.004	0.006	0.005	0.005
CV %	10.1	15.5	16.2	13.9

NIBSC 0.1 ng/ml (N = 16)

Mean values	1 st run	2 nd run	3 rd run	Average
OD _{450nm}	0.133	0.121	0.118	0.124
Std. Deviation	0.019	0.008	0.011	0.013
CV %	14.1	6.9	9.2	10.1
S/Co	1.5	1.4	1.4	1.4

Lot # 2

Negative Sample (N = 16)

Mean values	1 st run	2 nd run	3 rd run	Average
OD _{450nm}	0.034	0.035	0.031	0.033
Std. Deviation	0.005	0.005	0.005	0.005
CV %	15.5	13.5	15.4	14.8

NIBSC 0.1 ng/ml (N = 16)

Mean values	1 st run	2 nd run	3 rd run	Average
OD _{450nm}	0.125	0.129	0.129	0.127
Std. Deviation	0.012	0.010	0.013	0.012
CV %	9.7	7.5	10.4	9.2
S/Co	1.5	1.5	1.6	1.5

Lot # 3

Negative Sample (N = 16)

Mean values	1 st run	2 nd run	3 rd run	Average
OD _{450nm}	0.034	0.035	0.034	0.034
Std. Deviation	0.004	0.005	0.004	0.004
CV %	10.5	12.9	11.2	11.5

NIBSC 0.1 ng/ml (N = 16)

Mean values	1 st run	2 nd run	3 rd run	Average
OD _{450nm}	0.124	0.129	0.126	0.126
Std. Deviation	0.009	0.012	0.015	0.012
CV %	7.2	9.4	11.7	9.4
S/Co	1.5	1.5	1.5	1.5

The variability shown in the tables did not result in sample misclassification.

Appendix III



HCV Ab
Enzyme Immunoassay Test
Product N°:4250 (96 test)
Product N°:4251 (192 test)

▽ 96-192 Test

[IVD]

SUMMARY OF PROCEDURE

1. **STEP 1** Place 200 µl of Negative Control, Calibrator and Positive Control in the wells of the strips, leaving A1 well for the blank. Place 200 µl of Sample Diluent in the samples wells and then 10 µl of samples.
2. Dispense 50 µl of Assay Diluent in all the Control, Calibrator and samples wells. Check that the color of samples has turned to dark blue.
3. Incubate for 45 min. at 37°C
4. Wash the plate 4-5 times (350 µl).
5. **STEP 2** Place 100 µl of Enzyme Conjugate in each well except the A1 blank well.
6. Incubate for 45 min. at 37°C
7. Wash the plate 4-5 times (350 µl).
8. **STEP 3** Place 100 µl of Chromogen/Substrate in each well included A1.
9. Incubate for 15 min. at room temperature (18-30°C)
10. **STEP 4** Add 100 µl of Stop Solution
11. Read absorbance at 450 nm & 620 nm

INTENDED USE

Third Generation Enzyme Immunoassay for the qualitative detection of antibodies to Hepatitis C Virus (HCV Ab) in human serum (-). The kit may be used for the screening of blood units and the follow-up of HCV-infected patients.

For *in vitro* diagnostic use only

SUMMARY AND EXPLANATION

Hepatitis C is a viral infection of the liver which had been referred to as parenterally transmitted "non A, non B hepatitis" until identification of the causative agent in 1989. The discovery and characterization of the hepatitis C virus (HCV) led to the understanding of its primary role in post-transfusion hepatitis and its tendency to induce persistent infection¹⁻³.

HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year⁴.

HCV is spread primarily by direct contact with human blood. The major causes of HCV infection worldwide are use of unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized. No vaccine is currently available to prevent hepatitis C and treatment for chronic hepatitis C is too costly for most persons in developing countries to afford. Thus, from a global perspective, the greatest impact on hepatitis C disease burden will likely be achieved by focusing efforts on reducing the risk of HCV transmission from nosocomial exposures (e.g. blood transfusions, unsafe injection practices) and high-risk behaviours (e.g. injection drug use)^{5,7}.

Hepatitis C virus (HCV) is one of the viruses (A, B, C, D, and E), which together account for the vast majority of cases of viral hepatitis. It is an enveloped RNA virus in the *flaviviridae* family which appears to have a

narrow host range. Humans and chimpanzees are the only known species susceptible to infection, with both species developing similar disease.

An important feature of the virus is the relative mutability of its genome, which in turn is probably related to the high propensity (80%) of inducing chronic infection. HCV is clustered into several distinct genotypes which may be important in determining the severity of the disease and the response to treatment⁸⁻¹².

The incubation period of HCV infection before the onset of clinical symptoms ranges from 15 to 150 days. In acute infections, the most common symptoms are fatigue and jaundice; however, the majority of cases (between 60% and 70%), even those that develop chronic infection, are asymptomatic. About 80% of newly infected patients progress to develop chronic infection. Cirrhosis develops in about 10% to 20% of persons with chronic infection, and liver cancer develops in 1% to 5% of persons with chronic infection over a period of 20 to 30 years. Most patients suffering from liver cancer who do not have hepatitis B virus infection have evidence of HCV infection. The mechanisms by which HCV infection leads to liver cancer are still unclear. Hepatitis C also exacerbates the severity of underlying liver disease when it coexists with other hepatic conditions. In particular, liver disease progresses more rapidly among persons with alcoholic liver disease and HCV infection. HCV is spread primarily by direct contact with human blood. Transmission through blood transfusions that are not screened for HCV infection, through the reuse of inadequately sterilized needles, syringes or other medical equipment, or through needle-sharing among drug-users, is well documented. Sexual and perinatal transmission may also occur, although less frequently. Other modes of transmission such as social, cultural, and behavioural practices using percutaneous procedures (e.g. ear and body piercing, circumcision, tattooing) can occur if inadequately sterilized equipment is used. HCV is not spread by sneezing, hugging, coughing, food or water, sharing eating utensils, or casual contact¹³⁻¹⁶.

In both developed and developing countries, high risk groups include injecting drug users, recipients of unscreened blood, haemophiliacs, dialysis patients and persons with multiple sex partners who engage in unprotected sex. In developed countries, it is estimated that 90% of persons with chronic HCV infection are current and former injecting drug users and those with a history of transfusion of unscreened blood or blood products. In many developing countries, where unscreened blood and blood products are still being used, the major means of transmission are unsterilized injection equipment and unscreened blood transfusions. In addition, people who use traditional scarification and circumcision practices are at risk if they use or re-use unsterilized tools¹⁷.

WHO estimates that about 170 million people, 3% of the world's population, are infected with HCV and are at risk of developing liver cirrhosis and/or liver cancer. The prevalence of HCV infection in some countries in Africa, the Eastern Mediterranean, South-East Asia and the Western Pacific (when prevalence data are available) is high compared to some countries in North America and Europe¹⁸⁻²².

Diagnostic tests for HCV are used to prevent infection through screening of donor blood and (-), to establish the clinical diagnosis and to make better decisions regarding medical management of a patient. Diagnostic tests commercially available today are based on Enzyme immunoassay (EIA) for the detection of HCV specific antibodies. EIAs can detect more than 95% of chronically infected patients but can detect only 50% to 70% of acute infections. A recombinant immunoblot assay (RIBA) that identifies antibodies which react with individual HCV antigens is often used as a supplemental test for confirmation of a positive EIA result. Testing for HCV circulating by amplification tests RNA (e.g. polymerase chain reaction or PCR, branched DNA assay) is also being utilized for confirmation of serological results as well as for assessing the effectiveness of antiviral therapy. A positive result indicates the presence of active infection and a potential for spread of the infection and/or the development of chronic liver disease²³⁻²⁶.

Antiviral drugs such as interferon taken alone or in combination with ribavirin, can be used for the treatment of persons with chronic hepatitis C, but the cost of treatment is very high. Treatment with interferon alone is effective in about 10% to 20% of patients. Interferon combined with ribavirin is effective in about 30% to 50% of patients. Ribavirin does not appear to be effective when used alone²⁷.

There is no vaccine against HCV. Research is in progress but the high mutability of the HCV genome complicates vaccine development. Lack of knowledge of any protective immune response following HCV infection also impedes vaccine research. It is not known whether the immune system is able to eliminate the virus²⁸⁻²⁹.

Some studies, however, have shown the presence of virus neutralizing antibodies in patients with HCV infection. In the absence of a vaccine, all precautions to prevent infection must be taken including (a) screening and testing of blood and organ donors; (b) Virus inactivation of (-) derived products; (c) implementation and maintenance of infection control practices in health care settings, including appropriate sterilization of medical and dental equipment; (d) promotion of behaviour change among the general public and health care workers to reduce overuse of injections and to use safe injection practices; and (e) Risk reduction counselling for persons with high-risk drug and sexual practices³⁰.

The genome encodes for structural components, a nucleocapsid protein and two envelope glycoproteins, and functional constituents involved in the virus replication and protein processing.

The nucleocapsid-encoding region seems to be the most conservative among the isolates obtained all over the world.

PRINCIPLE OF THE PROCEDURE

Microplates are coated with HCV-specific antigens derived from "core" and "ns" regions encoding for conservative and immunodominant antigenic determinants (Core peptide, recombinant NS3, NS4 and NS5 peptides).

The solid phase is first treated with the diluted sample and HCV Ab are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound HCV antibodies, IgG and IgM as well, are detected by the addition of polyclonal specific anti hlgG/IgM antibodies, labelled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HCV antibodies present in the sample. A cut-off value let optical densities be interpreted into HCV antibody negative and positive results.

REAGENTS

Code	4250	4251
Test N°	96	192
Microplate	1	2
Negative Control	2x1,8 ml	3x1,8 ml
Positive Control	1x1,8 ml	2x1,8 ml
Calibrator	1 lyo	2 lyo
Wash Buffer 20x concentrate	1x60 ml	2x60 ml
Enzyme Conjugate	1x 16 ml	1x32 ml
Chromogen Substrate	1x20 ml	1x40 ml
Assay Diluent	1x10 ml	1x20 ml
Stop Solution	1x20 ml	1x40 ml
Sample Diluent	1x50 ml	2x50 ml

Bring to room temperature before use

Antigen Coated Wells (Microplate)

Microplate with breakable wells coated with Core peptide, recombinant NS3, NS4 and NS5 peptides, and sealed into a bag with desiccant.

Negative Control

Ready to use reagent, with 0,5% Casein 10% Glycerol 25 mM Na-citrate buffer pH 4,8+/-0.1, 0,5% NP 40, 1mM DTT and 0.1% Kathon GC as preservative. The

Negative Control is olive green colour coded.

Positive Control

Ready to use reagent, with 2% BSA, anti-HCV human antibodies, 100 mM Tris HCL buffer pH 7.4 +/-0.1, and 0.1% Kathon GC as preservative. The Positive Control is dark green colour coded.

Important Note: Even if this material has been chemically inactivated, handle as potentially infectious.

Calibrator

Lyophilized reagent. To be reconstituted with the volume of EIA grade water reported on the label. It contains 4% bovine serum proteins, human anti-HCV antibodies whose content is calibrated on the NIBSC Working Standard code 99/588-003-WI, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

Note: The volume necessary to reconstitute the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

Important Note: Even if this material has been chemically inactivated, handle as potentially infectious.

Wash buffer 20X concentrate

20X concentrated solution. Once diluted, the working solution contains 10 mM phosphate buffer pH 7.0±0.2, 0.05% Tween 20 and 0.1% Kathon GC.

Enzyme Conjugate

Ready to use and red colour coded Horseradish Peroxidase (HRP) conjugated to goat polyclonal antibodies to human IgG and IgM, MOPS buffer pH 6.2 - 6.7. Contains bovine protein preserved with 0.02% methylisothiazolone and bromonitrodioxane, 20 ppm Proclin 300.

Chromogen Substrate

Ready to use 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methylbenzidine (TMB) and 0.02% hydrogen peroxide (H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

Assay Diluent

Ready to use 10 mM Tris buffered solution pH 7.8 +/-0.1, 50% Goat serum, 0.09% Na-azide and 0.1% Kathon GC as preservatives for the pre-treatment of samples and Controls in the plate, blocking interference.

Note: Use all the content of one vial before opening a second one. The reagent is sensitive to oxidation.

Stop Solution

Ready to use 0.3 M H₂SO₄ solution.

Sample Diluent

Ready to use 0,5% Casein, 10% Glycerol 25 mM Na-citrate buffer pH 4,8+/-0.1 0,5% NP 40, 1mM DTT and 0.1% Kathon GC as preservative. To be used to dilute the sample.

Note: The diluent changes colour from olive green to dark bluish green in the presence of sample.

Adhesive sealing foils

OTHER MATERIALS REQUIRED

Calibrated Micropipettes (100 µl and 50 µl) and disposable plastic tips.
EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
Timer with 60 minute range or higher.
Absorbent paper tissues.
Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (±1°C tolerance).
Calibrated ELISA microwell reader with 450 nm (reading) and possibly with 620-630 nm (blanking) filters.
Calibrated ELISA microplate washer.
Vortex or similar mixing tools.

STORAGE AND STABILITY OF REAGENTS

Reagents must be stored at 2/8°C.
The expiry date is printed on each component and on the box label.
Reagents have a limited stability after opening and/or preparation.

Microplate Use: open the package on the opposite end from the code which is useful for identification purposes, remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the dessiccant, expell the air and seal by pressing the closure.

Negative Control Ready to use. Mix well on vortex before use.

Positive Control Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.

Calibrator Reconstitute carefully the content of the lyophilized vial with the volume of EIA grade water reported on its label. Mix well on vortex before use. Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.

Note: When reconstituted the Calibrator is not stable. Store in aliquots at -20°C.

Wash buffer concentrate The whole content of the concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

Note: Once diluted, the Wash solution is stable for 1 week at +2..8° C.

Enzyme Conjugate Ready to use. Mix well on vortex before use. Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

Sample Diluent Ready to use. Mix well on vortex before use.

Chromogen Substrate Ready to use. Mix well on vortex before use. Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces. If this component has to be transferred use only plastic and, if possible, sterile disposable container.

Assay Diluent Ready to use. Mix well on vortex before use.

Stop Solution Ready to use. Mix well on vortex before use.

WARNINGS AND PRECAUTIONS

For in vitro Diagnostic Use Only.

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.

2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.

3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.

5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.

6. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.

7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.

8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.

9. Avoid cross-contamination between serum/(-) samples by using disposable tips and changing them after each sample.

10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.

11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.

12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.

14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..

15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

17. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.

18. The ELISA incubator has to be set at +37°C (tolerance of ±1°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

19. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 µl/well of washing

solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

20. Incubation times have a tolerance of $\pm 5\%$.

21. The microplate reader has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630 nm) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 2.0 ; repeatability $\geq 1\%$. Blanking is carried out on the well identified in the section "ASSAY PROCEDURE". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.

22. When using ELISA automated workstations, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "QUALITY CONTROL".

The assay protocol has to be installed in the operating system of the unit and validated by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing. The carry over effect must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.

23. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2-8°C, firmly capped.

SPECIMEN COLLECTION

1. Blood is drawn aseptically by venepuncture and (-) serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.

3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.

4. Haemolysed (red) and lipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as well as they could give rise to false results.

5. Sera and (-) can be stored at +2°-8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen sample should not be frozen/thawed more than once as this may generate particles that could affect the test result.

6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8 μ m filters to clean up the sample for testing.

PROCEDURE

PREASSAY PROCEDURE

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of

it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.

3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Reconstitute the Calibrator as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Automated assay

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 200 μ l Sample Diluent and then 10 μ l sample.

All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.

Do not dilute controls/calibrator as they are ready to use.

Dispense 200 μ l controls/calibrator in the appropriate control/calibration wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual Assay

1. Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
2. Dispense 200 μ l of Negative Control in triplicate, 200 μ l Calibrator in duplicate and 200 μ l Positive Control in single in proper wells. Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !
3. Add 200 μ l of Sample Diluent to all the sample wells; then dispense 10 μ l sample in each properly identified well. Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.
4. Dispense 50 μ l Assay Diluent into all the controls/calibrator and sample wells. Check that the color of samples has turned to dark blue.
5. Incubate the microplate for 45 min at +37°C.
6. Wash the microplate with an automatic washer by delivering and aspirating 350 μ l/well of diluted washing solution as reported previously (section WARNINGS AND PRECAUTIONS).
7. Pipette 100 μ l Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.
8. Incubate the microplate for 45 min at +37°C.
9. Wash microwells as in step 6.
10. Pipette 100 μ l Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature for 15 minutes.
11. Pipette 100 μ l Stop Solution into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction.

Addition of acid will turn the positive control and positive samples from blue to yellow.

- Measure the colour intensity of the solution in each well, as described in section WARNINGS AND PRECAUTIONS, at 450 nm filter (reading) and possibly at 620-630nm (background subtraction), blanking the instrument on A1.

An example of dispensation scheme is reported below:

Microplate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank; NC= Negative Control; CAL = Calibrator; PC= Positive Control; S = Samples

IMPORTANT NOTES

- Visually monitor that samples have been diluted and dispensed into appropriate wells. This is simply achieved by checking that the colour of dispensed samples has turned to dark bluish-green while the colour of Negative Control has remained olive green.
- Check that the colour of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.
- Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.
- Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.
- Do not expose to strong direct illumination. High background might be generated.
- If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.
- Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.
- Shaking at 350 \pm 150 rpm during incubation has been proved to increase the sensitivity of the assay of about 20%.

QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the expected OD_{450nm} or S/Co values have been matched in the analysis.

Control that the following data are matched:

Blank well (Bw):	OD _{Bw} < 0.100;
Negative Control (NC)	OD _{NC} after blanking < 0.050;
Calibrator (CAL)	S/Co > 1.1;
Positive Control (PC)	OD _{PC} > 1.000;

If the results of the test match the requirements stated above, proceed to the next section.

TROUBLESHOOTING

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well: OD _{450nm} > 0.100	1. that the Chromogen/Substrate solution has not become contaminated during the assay.
Negative Control after blanking:	1. that the washing procedure and the washer settings are as validated

OD _{450nm} > 0.050	in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control); 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator: S/Co < 1.1	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of a wrong reagent); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control OD _{450nm} < 1.000	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD _{450nm} value > 0.150, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

RESULTS

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD_{450nm} value of the Negative Control (NC):

$$\text{Cut-Off (Co)} = \text{NC} + 0.350$$

The value found for the test is used for the interpretation of results as described in the next paragraph.

Important note: When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.

INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD_{450nm} and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HCV or that the blood unit may be transfused.

Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. The blood unit should not be transfused.

A positive result is indicative of HCV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.
2. Any positive result should be confirmed by an alternative method capable to detect IgG and IgM antibodies (confirmation test) before a diagnosis of viral hepatitis is formulated.
3. As proved in the Performance Evaluation of the product, the assay is able to detect seroconversion to anti HCV core antibodies earlier than some other commercial kits. Therefore a positive result, not confirmed with these commercial kits, does not have to be ruled out as a false positive result! The sample has to be anyway submitted to a confirmation test (supplied upon request by Delta Biologicals S.r.l.).
4. As long as the assay is able to detect also IgM antibodies some discrepant results with other commercial products for the detection of anti HCV antibodies - lacking anti hIgM conjugate in the formulation of the enzyme tracer and therefore missing IgM reactivity - may be present. The real positivity of the sample for antibodies to HCV should be then confirmed by examining also IgM reactivity, important for the diagnosis of HCV infection.
5. When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.
6. Diagnosis of viral hepatitis infection has to be done and released to the patient only by a qualified medical doctor.

An example of calculation is reported below:

The following data must not be used instead of real figures obtained by the user.

Negative Control: 0.019 – 0.020 – 0.021 OD_{450nm}
Mean Value: 0.020 OD_{450nm}
Lower than 0.050 Accepted

Positive Control: 2.189 OD_{450nm}
Higher than 1.000 Accepted

Cut-Off 0.020+0.350 = 0.370

Calibrator: 0.550 - 0.530 OD_{450nm}
Mean value: 0.540 OD_{450nm}
S/Co 1.4
S/Co higher than 1.1 Accepted

Sample 1: 0.070 OD_{450nm}
Sample 2: 1.690 OD_{450nm}
Sample 1 S/Co < 0.9; negative
Sample 2 S/Co > 1.1; positive

PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

Limit of detection

The limit of detection of the assay has been calculated by means of the British Working Standard for anti-HCV, NIBSC code 99/588-003-WI. The table below reports the mean OD_{450nm} values of this standard when diluted in negative plasma and then examined.

Dilution factor	Lot # 1 S/Co	Lot # 2 S/Co
1 X	2.0	2.0
2 X	1.1	1.2
4 X	0.7	0.8
8 X	0.5	0.5
Negative plasma	0.3	0.3

In addition the sample coded Accurun 1 – series 3000 - supplied by Boston Biomedica Inc., USA, has been evaluated "in toto" showing the results below:

HCV Ab Lot ID	Accurun 1 Series	S/Co
1201	3000	1.5
0602	3000	1.5
1202	3000	1.9

In addition, n° 7 samples, tested positive for HCV Ab with Ortho HCV 3.0 S/Co, code 930820, lot. # EXE065-1, were diluted in HCV Ab negative plasma in order to generate limiting dilutions and then tested again on HCV Ab lot. # 1202, and Ortho.

The following table reports the data obtained.

Sample n°	Limit Dilution	HCV Ab S/Co	Ortho 3.0 S/Co
1	256 X	1.9	1.3
2	256 X	1.9	0.7
3	256 X	2.4	1.0
4	128 X	2.5	3.2
5	85 X	3.3	1.4
6	128 X	2.2	0.8
7	135 X	3.2	2.2

DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The Performance Evaluation of the device was carried out in a trial conducted on more than total 5000 samples.

Diagnostic specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. A total of more 5000 unselected donors, including 1st time donors, were examined.

The diagnostic specificity was assessed against a kit US FDA approved.

5043 blood donors were tested providing a specificity of 99.5%.

210 hospitalized patients were tested for HCV Ab; a diagnostic specificity of 99.5% was found.

Moreover, diagnostic specificity was assessed by testing 162 potentially interfering specimens (other infectious diseases, *E. coli* antibody positive, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolyzed, lipemic, etc.). A value of specificity of 100% was assessed.

No false reactivity due to the method of specimen preparation has been observed. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.

Frozen specimens have been tested, as well, to check for interferences due to collection and storage.

No interference was observed.

Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.

The diagnostic sensitivity has been assessed externally on a total number of 359 specimens; a diagnostic sensitivity of 100% was found. Internally more than other 50 positive samples were tested, providing a value of diagnostic sensitivity of again 100%.

Positive samples from infections carried out by different genotypes of HCV were tested as well.

Furthermore, most of seroconversion panels available from Boston Biomedica Inc., have been studied.

Results are reported below for some of them.

Appendix IV



GPT (ALAT) IFCC FLUID (5+1)

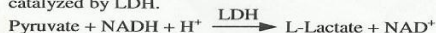


Kinetic determination of Glutamate pyruvate transaminase - IFCC Version without pyridoxal phosphate activation

MEASUREMENT PRINCIPLE



The increase in pyruvate is determined in an indicator reaction catalyzed by LDH.



NADH is oxidized to NAD. The rate of the NADH decrease is directly proportional to the rate of formation of pyruvate and thus the GPT activity.

CONCENTRATION OF WORKING SOLUTION

Buffer	
TRIS-Buffer pH 7.5 (25°C)	70.00 mmol/l
L-Alanine	410.00 mmol/l
LDH	≥ 1.7 U/ml
Starter	
NADH	0.3 mmol/l
α-Ketoglutarate	18 mmol/l

STORAGE AND STABILITY

The sealed reagents are stable up to the indicated expiry date if stored at +2° - +8°C.

INTENDED USE

In vitro test for the quantitative determination of GPT (ALAT) in human serum and plasma.

PRECAUTIONS AND WARNINGS

For in vitro diagnostic use only. Attend to the normal precautions required for handling all laboratory reagents. The reagents contain sodium azide as preservative. Do not swallow. Avoid contact with skin and mucous membranes.

EXPECTED VALUES

37°C	U/l	μkat/l
male	up to 41	up to 0.70
female	up to 31	up to 0.50

SAMPLE MATERIAL

Serum, Heparin-/EDTA-Plasma

QUALITY CONTROL

Centronorm, Centropath or all other control sera with values determined by this method may be employed.

LINEARITY

280 U/l at 334/340 nm

Changes of absorbance without the measurement range should be repeated by dilution of 20 μl sample with 200 μl phys. NaCl (0.9%) solution. In this case multiply the result by 11.

CONVERSION FACTOR

U/l ⇔ μkat/l Factor: 0.0167
μkat/l ⇔ U/l Factor: 60

TEST SPECIFICATIONS

Reproducibility in Intraassay and Interassay on Hitachi 717:

Sample	Within run (n= 25)			Between run (n= 17)		
	Mean U/l	SD U/l	%CV	Mean U/l	SD U/l	%CV
Level 1	35.6	0.83	2.34	39.2	0.67	1.71
Level 2	120.1	1.33	1.11	82.2	1.62	1.97
Level 3	178.4	2.53	1.42	143.0	2.18	1.52

Edition 04/2013, TV-045-CE-003

TEST COMPARISON

Comparison with a test of the market leader on the Hitachi 717: n = 60; r = 0.9992; y = 1.0202x - 1.2293

ASSAY PROCEDURE

Wavelength : 334/340/365 nm
Temperature : 37° C

Reaction mixture (only for start with serum): Mix reagent 1 (5 volumes) with reagent 2 (1 volume) and temperate before use.

Stability of reaction mixture:

at + 2° - + 8°C: 15 days
at +18° - +22°C: 3 days

Pipette into cuvette:

	Start with serum
Reaction mixture (37°C)	1000 μl
Sample	100 μl
	Start with R2:
Buffer R1	1000 μl
Sample	100 μl
Reagent 2	200 μl

Mix and incubate for 30 sec. and measure the changes of absorbance for at least 3 min.

Determine the differences in absorbance/min. (ΔA/min)

CALCULATION

	Factor for serum start		
	334 nm	340 nm	365 nm
F x ΔA/min (U/l)	1780	1746	3235
F x ΔA/min (μkat/l)	29.67	29.11	53.93

	Factor for substrate start R2		
	334 nm	340 nm	365 nm
F x ΔA/min (U/l)	2103	2063	3823
F x ΔA/min (μkat/l)	35.06	34.39	63.73

APPLICATIONS

Applications are available for many analyzers after request

LITERATURE

Z. klin. Chem. u. klin. Biochem. 8 (1970) 658 and 10 (1972), 182

PACKAGE SIZES

KIT SIZE	ORDER NO.
2 x 60 ml	manual GF05000060
1 x 120 ml	manual GF05R05060
4 x 120 ml	manual GF05000120
10 x 60 ml	for Hitachi GF05911060
	704/902/717/911
5 x 120 ml	for Hitachi 717/911 GF05911120
5 x 120 ml	for Hitachi 911 barc. GF05911120-B
6 x 84 ml	for Hitachi 917/Modular-P GF05917084-B
6 x 84 ml	for Olympus AU GF05917084
8 x 30 ml	for Selectra GF05SEL030

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e-mail: info@centronic-gmbh.com
web: www.centronic-gmbh.com

Appendix V



GOT (ASAT) FLUID (5+1)

IVD



ALBION Kinetic determination of Glutamate oxaloacetate transaminase - IFCC Version without pyridoxal phosphate activation

MEASUREMENT PRINCIPLE

α -Ketoglutarate + L-Aspartate $\xrightarrow{\text{GOT}}$ L-Glutamate + Oxaloacetate
The increase in oxaloacetate is determined in an indicator reaction catalyzed by MDH.

Oxaloacetate + NADH + H⁺ $\xrightarrow{\text{MDH}}$ L-Malate + NAD⁺

NADH is oxidized to NAD. The rate of the NADH decrease is directly proportional to the rate of formation of oxaloacetate and thus the GOT activity.

CONCENTRATION OF WORKING SOLUTION

Buffer	
TRIS-Buffer pH 7.8 (30°C)	80.00 mmol/l
L-Aspartate	200.00 mmol/l
LDH	≥ 1.6 U/ml
MDH	≥ 0.5 U/ml
Starter	
NADH	0.18 mmol/l
α -Ketoglutarate	12 mmol/l

STORAGE AND STABILITY

The sealed reagents are stable up to the indicated expiry date if stored at +2° - +8°C.

INTENDED USE

In vitro test for the quantitative determination of GOT (ASAT) in human serum and plasma.

PRECAUTIONS AND WARNINGS

For in vitro diagnostic use only. Attend to the normal precautions required for handling all laboratory reagents. The reagents contain sodium azide as preservative. Do not swallow. Avoid contact with skin and mucous membranes.

EXPECTED VALUES

37°C	U/l	μkat/l
male	up to 38 U/l	up to 0.63
female	up to 32 U/l	up to 0.53

SAMPLE MATERIAL

Serum, Heparin-/EDTA-Plasma

QUALITY CONTROL

Centronorm, Centropath or all other control sera with values determined by this method may be employed.

LINEARITY

280 U/l at 334/340 nm

For higher values the measurement range should be repeated by dilution of 20 μl sample with 200 μl phys. NaCl solution. In this case multiply the result by 11.

CONVERSION FACTOR

U/l \Rightarrow μkat/l Factor: 0.0167
μkat/l \Rightarrow U/l Factor: 60

TEST SPECIFICATIONS

Reproducibility in Intraassay and Interassay on Hitachi 717:

	Within run (n= 30)			Between run (n= 18)		
Sample	Mean U/l	SD U/l	%CV	Mean U/l	SD U/l	%CV
Level 1				33.5	0.90	2.69
Level 2	115.7	1.48	1.28	90.1	1.59	1.76
Level 3	192.5	1.22	0.64	204.1	5.38	2.63

TEST COMPARISON

Comparison with a test of the market leader on the Hitachi 717:
n = 60; r = 0.9996; y = 1.04x - 2.0549

ASSAY PROCEDURE

Wavelength : 334/340/365 nm
Temperature : 37° C

Reaction mixture (only for start with serum): Mix reagent 1 (5 volumes) with reagent 2 (1 volume) and temperate before use.

Stability of reaction mixture:

at + 2° - + 8°C: 15 days
at +18° - +22°C: 3 days

Pipette into cuvette:

Start with serum
React.mix. 1000 μl
Sample 100 μl

Start with R2
Reagent 1 1000 μl
Sample 100 μl
Reagent 2 200 μl

Mix and incubate for 30 sec. and measure the changes of absorbance for at least 3 min..

Determine the differences in absorbance/min. (ΔA/min)

CALCULATION

	Factor for serum start		
	334 nm	340 nm	365 nm
F x ΔA/min (U/l)	1780	1746	3235
F x ΔA/min (μkat/l)	29.67	29.11	53.93

	Factor for substrate start R2		
	334 nm	340 nm	365 nm
F x ΔA/min (U/l)	2103	2063	3823
F x ΔA/min (μkat/l)	35.06	34.39	63.73

APPLICATIONS

Applications are available for many analyzers after request

LITERATURE

Z. klin. Chem. u. klin. Biochem. 8 (1970) 658 and 10 (1972), 182

PACKAGE SIZES

KIT SIZE		ORDER NO.
2 x 60 ml	manual	GF04000060
1 x 120 ml	manual	GF04R05060
4 x 120 ml	manual	GF04000120
10 x 60 ml	for Hitachi	GF04911060
	704/902/717/911	
5 x 120 ml	for Hitachi 717/911	GF04911120
5 x 120 ml	for Hitachi 911 barc.	GF04911120-B
6 x 84 ml	for Hitachi 917/	GF04917084-B
	Modular-P	
6 x 84 ml	for Olympus AU	GF04917084
8 x 30 ml	for Selectra	GF04SEL030

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Appendix VI



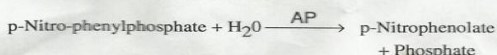
ALKALINE PHOSPHATASE-FLUID (5+1) DGKC

IVD



Optimized Determination of Alkaline Phosphatase in Body Fluids (DGKC Method)

MEASUREMENT PRINCIPLE



The p-Nitrophenol is present as yellow coloured p-Nitrophenolate. The increase of absorbance per minute at 405 nm is proportional to the enzyme activity.

CONCENTRATION OF WORKING SOLUTION

Buffer
Diethanolamin (pH=10.0) 0.6 mol/l
MgCl₂ 0.4 mmol/l
Starter
p-Nitrophenylphosphate 7 mmol/l

STORAGE AND STABILITY

The sealed reagents are stable up to the indicated expiry date if stored at +2° - +8°C.

INTENDED USE

In vitro test for the quantitative determination of Alkaline phosphatase in human serum and plasma.

PRECAUTIONS AND WARNINGS

For in vitro diagnostic use only. Attend to the normal precautions required for handling all laboratory reagents. Buffer reagent contain Diethanolamin. In the case of contact rinse affected area with plenty of water; get immediate medical attention after contact with eyes or if ingested.

EXPECTED VALUES

(in serum, Heparin plasma) at 37 °C

		U/l	µkat/l
Children:		180 - 1200	1.3 - 20
Adults:	male:	100 - 270	1.67 - 4.50
	female:	100 - 240	1.67 - 4.00

SAMPLE MATERIAL

Serum, Heparin Plasma. Hemolysis will interfere. The values in serum controls increase directly after reconstitution: Measure controls after reconstitution after 1 hour at +20°C

QUALITY CONTROL

Centronorm, Centropath or all other control sera with values determined by this method may be employed.

LINEARITY

935 U/l

At higher values repeat the test using a sample diluted 1 + 9 with 0.9% NaCl. Multiply the result by 10.

CONVERSION FACTOR

U/l ⇔ µkat/l Factor: 0.0167
µkat/l ⇔ U/l Factor: 60

TEST SPECIFICATIONS

Reproducibility in Intraassay and Interassay on Hitachi 717:

	Within run (n= 30)			Between run (n= 30)		
Sample	Mean U/l	SD U/l	%CV	Mean U/l	SD U/l	%CV
Level 1	70.8	1.54	2.18			
Level 2	277.4	2.75	0.99	276	4.84	1.75
Level 3	525	6.01	1.14	484.7	12.68	2.62

TEST COMPARISON

Comparison with another commercial available test on the Hitachi 717:

n = 58; r = 0.9986; y = 0.9983x - 1.7311

ASSAY PROCEDURE

Wavelength : 405 nm
Temperature : 37° C

Stability of reaction mixture:

at + 2° - + 8°C: 25 days
at +18° - +22°C: 5 days

Pipette into cuvette:

Start with serum

Reaction mixture (only for start with serum): Mix reagent 1 (5 volumes) with reagent 2 (1-volume) and temperate before use.

Reaction mixture 1000 µl
Sample 20 µl

Start with R2:

Buffer R1 1000 µl
Sample 20 µl
Reagent 2 200 µl

Mix and incubate for 30 sec. and measure the changes of absorbance for at least 3 min.

Determine the differences in absorbance/min. (ΔA/min)

CALCULATION

	Factor for serum start
F x ΔA/min (U/l)	2757
F x ΔA/min (µkat/l)	45,9

	Factor for substrate start R2
F x ΔA/min (U/l)	3298
F x ΔA/min (µkat/l)	54,9

APPLICATIONS

Applications are available for many analyzers after request

LITERATURE

Thomas, L.: Labor u. Diagnostik, 1. Aufl., S. 64, Med. Verlag, Marburg, (1978)
Weishaar, D., Grossrau, E. u. Faderl, B.: Mediz. Welt 26, 5.387-390, (1975)

PACKAGE SIZES

KIT SIZE	ORDER NO.
2 x 60 ml manual	AF02000060
1 x 120 ml manual	AF02R05060
4 x 120 ml manual	AF02000120
10 x 60 ml for Hitachi	AF02911060
704/902/717/911	
5 x 120 ml for Hitachi 717/911	AF02911120
5 x 120 ml for Hitachi 911 barc.	AF02911120-B
6 x 84 ml for Hitachi 917/Modular-P	AF02917084-B
6 x 84 ml for Olympus AU	AF02917084
8 x 30 ml for Selectra	AF02SEL030

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Edition 04/2013, TV-004-CE-003

Appendix VII

12000000001 V15.0

TP

Total Protein



C Corrosive

R 35 Causes severe burns.

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336

Reagent handling

R1: Ready for use

R2: Ready for use

(Users of Roche/Hitachi 902 analyzer: Predilute R1 1 + 2 with distilled/deionized water for application TPDIL.)

Absorption of atmospheric CO₂ by the opened reagent bottles leads to impaired calibration stability. This kit therefore requires the use of color-coded chimneys which reduce the uptake of CO₂ by the reagents. The chimneys should be placed directly into the appropriate reagents: white for R1, black for R2. The chimneys can be reused for reagent bottles within the same kit. However, to avoid contamination of the reagent with detergent or dilution of the reagent with water it is not permitted to wash the chimneys before reuse. Chimneys are used on all systems.

Storage and stability

Unopened kit components: Up to the expiration date at 15-25 °C

R1: 4 weeks opened and refrigerated on the analyzer

R2: 4 weeks opened and refrigerated on the analyzer

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum

Plasma: Li-/Na-/NH₄⁺-heparin or EDTA-plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:^{3,4,5} 1 month at 2-8 °C

6 months at (-15)-(-25) °C

The total protein concentration is 0.4 to 0.8 g/dL lower when the sample is collected from a patient situated in the recumbent position rather than upright.⁶

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section
- 0.9 % NaCl
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

cobas[®]

Calibration

Traceability: This method has been standardized against SRM 927.

S1: 0.9 % NaCl

S2: C.f.a.s.

Calibration frequency

2-point calibration is recommended:

- after reagent lot change
- as required following quality control procedures

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

Use the assigned value for total protein according to the reference method (for Germany) or with sample blank.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample.

Conversion factor: g/dL x 10 = g/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Icterus:⁷ No significant interference up to an I index of 21 for conjugated bilirubin and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 359 µmol/L or 21 mg/dL).

Hemolysis:⁷ No significant interference up to an H index of 650 (approximate hemoglobin concentration: 404 µmol/L or 650 mg/dL). Interference results from the fact that hemoglobin is reacting as a protein in the Total Protein assay.

Lipemia (Intralipid):⁷ No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Dextran up to concentrations of 30 mg/mL does not interfere.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{8,9}

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.¹⁰

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi analyzers. Refer to the latest version of the carry-over evasion lists and the operator's manual for further instructions. US users refer to the Special Wash Programming document (located on MyLabOnline website) and the operator's manual for special wash instructions.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

Measuring range: 0.2-15 g/dL (2.0-150 g/L)

Determine samples having higher concentrations via the rerun function. On instruments without rerun function, manually dilute samples having higher concentrations with 0.9 % NaCl or distilled/deionized water (e.g. 1 + 2). Multiply the result by the appropriate factor (e.g. 3).

Roche/Hitachi MODULAR analyzers:

Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement




Appendix VIII

11965042001V16

ALB plus

Albumin BCG method



Cat. No.	Bottle	Contents	Indicates Roche/Hitachi analyzer(s) on which kit(s) can be used			
			902	912	917	MODULAR
11970909 216	1	REAGENT 6 x 64 mL				P
	2	REAGENT 6 x 16 mL				D
11929631 216	1	REAGENT 6 x 258 mL				
11970917 216	2	REAGENT 6 x 68 mL				
11929640 216	1	REAGENT 4 x 641 mL				
11970925 216	2	REAGENT 4 x 278 mL				
11970569 216	1	REAGENT 12 x 50 mL				
	2	REAGENT 6 x 22 mL				

Some analyzers and kits shown may not be available in all countries. For additional system applications, contact your local Roche Diagnostics representative.

English

System information
For Roche/Hitachi 912/917/MODULAR analyzers: ACN 413.

Intended use
In vitro test for the quantitative determination of albumin in human serum and plasma on Roche automated clinical chemistry analyzers.

Summary^{1,2}
Albumin is a carbohydrate-free protein, which constitutes 55-65 % of total plasma protein. It maintains oncotic plasma pressure, and is also involved in the transport and storage of a wide variety of ligands and is a source of endogenous amino acids. Albumin binds and solubilizes various compounds, e. g. bilirubin, calcium and long-chain fatty acids. Furthermore albumin is capable of binding toxic heavy metal ions as well as numerous pharmaceuticals, which is the reason why lower albumin concentrations in blood have a significant effect on pharmacokinetics. Hyperalbuminemia is of little diagnostic significance except in the case of dehydration. Hypoalbuminemia occurs during many illnesses and is caused by several factors: compromised synthesis due either to liver disease or as a consequence of reduced protein uptake; elevated catabolism due to tissue damage (severe burns) or inflammation; malabsorption of amino acids (Crohn's disease); proteinuria as a consequence of nephrotic syndrome; protein loss via the stool (neoplastic disease). In severe cases of hypoalbuminemia, the maximum albumin concentration of plasma is 2.5 g/dL. Due to the low osmotic pressure of the plasma, water permeates through blood capillaries into tissue (edema). The determination of albumin allows monitoring of a controlled patient dietary supplementation and serves also as an excellent test of liver function.

Test principle³
Colorimetric assay with endpoint method

- Sample and addition of R1
- Addition of R2 and start of the reaction:
At a pH value of 4.1 albumin displays a sufficiently cationic character to be able to bind with bromocresol green (BCG), an anionic dyestuff, to form a blue-green complex.

$$\text{albumin} + \text{BCG} \xrightarrow{\text{pH } 4.1} \text{albumin BCG complex}$$

The color intensity of the blue-green color is directly proportional to the albumin concentration and can be determined photometrically.

Reagents - working solutions

R1 Citrate buffer: 95 mmol/L, pH 4.1; preservative

R2 Citrate buffer: 95 mmol/L, pH 4.1; bromocresol green: 0.66 mmol/L; preservative

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling
R1: Ready for use
R2: Ready for use

Storage and stability
Unopened kit components: Up to the expiration date at 15-25 °C
R1: 28 days opened and refrigerated on the analyzer
R2: 28 days opened and refrigerated on the analyzer

Specimen collection and preparation
For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Heparin (Li-, Na-, NH₄⁺) or EDTA plasma.
Do not use fluoride plasma.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay.

Stability⁴

2.5 months at 15-25 °C
5 months at 2-8 °C
4 months at (-15)-(-25) °C

Materials provided
See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- Calibrators:
C.f.a.s. (Calibrator for automated systems), Cat. No. 10759350 190, 10759350 360 (for USA)
- Controls:
Precinorm U, Cat. No. 10171743 122,
Precinorm U plus, Cat. No. 12149435 122, 12149435 160 (for USA);
Precipath U, Cat. No. 10171778 122,
Precipath U plus, Cat. No. 12149443 122, 12149443 160 (for USA)
- 0.9 % NaCl
- General laboratory equipment

Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Calibration
Traceability: This method has been standardized against the CRM 470 reference preparation.

Appendix IX



05795800001V2.0

BILT3

Bilirubin Total Gen.3

Order information

REF	CONTENT	Analyzer(s) on which kit(s) can be used
05795265 190	Bilirubin Total Gen.3 ([1] 12 x 45 mL, [2] 6 x 20 mL)	Roche/Hitachi 902
05795320 190	Bilirubin Total Gen.3 ([1] 4 x 67 mL, [2] 4 x 16 mL)	Roche/Hitachi MODULAR P
05795338 190	Bilirubin Total Gen.3 ([1] 6 x 252 mL)	Roche/Hitachi MODULAR P, D
05795346 190	Bilirubin Total Gen.3 ([2] 6 x 63 mL)	Roche/Hitachi MODULAR P, D
05795354 190	Bilirubin Total Gen.3 ([1] 4 x 659 mL)	Roche/Hitachi MODULAR D
05795362 190	Bilirubin Total Gen.3 ([2] 4 x 144 mL)	Roche/Hitachi MODULAR D
10759350 190	Calibrator f.a.s. (12 x 3 mL) Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA) Code 401	
10171743 122	Precinorm U (20 x 5 mL) Code 300	
10171735 122	Precinorm U (4 x 5 mL) Code 300	
12149435 122	Precinorm U plus (10 x 3 mL) Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA) Code 300	
10171778 122	Precipath U (20 x 5 mL) Code 301	
10171760 122	Precipath U (4 x 5 mL) Code 301	
12149443 122	Precipath U plus (10 x 3 mL) Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA) Code 301	
10158046 122	Precibil (4 x 2 mL) Code 306	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL) Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL) Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL) Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL) Code 392	

Some analyzers and kits shown may not be available in all countries. For additional system applications, contact your local Roche Diagnostics representative.

English

System information

For Roche/Hitachi MODULAR analyzers: ACN 712

Intended use

In vitro test for the quantitative determination of total bilirubin in serum and plasma of adults and neonates on Roche automated clinical chemistry analyzers.

Summary¹

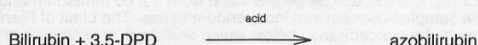
Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract.

Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

Test principle²

Colorimetric diazomethod

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium.



The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically.

Reagents - working solutions

R1 Phosphate: 25 mmol/L; detergents; stabilizers; pH 1.0

R2 3,5-dichlorophenyl diazonium salt: ≥ 1.35 mmol/L

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the European directive 1999/45/EC:



C Corrosive

R 35 Causes severe burns.

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336

Reagent handling

R1: Ready for use.

R2: Ready for use.

Storage and stability

Unopened kit components: up to the expiration date at 2-8 °C

R1: 6 weeks opened and refrigerated on the analyzer

R2: 6 weeks opened and refrigerated on the analyzer

Store the reagents protected from light.



BILT3

Bilirubin Total Gen.3

cobas®

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum

Plasma: Li-heparin, K₂-, K₃-EDTA plasma

(The use of EDTA-plasma with elevated hematocrit may lead to slightly lower values.)

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability: ^{a,3}	1 day at 15-25 °C
	7 days at 2-8 °C
	6 months at (-15)-(-25) °C

a) If care is taken to prevent exposure to light

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section
- 0.9 % NaCl
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Calibration

Traceability: The method was standardized against the Doumas method.⁴

S1: 0.9 % NaCl

S2: C.f.a.s.

Calibration frequency

2-point calibration is recommended:

- after reagent lot change
- as required following quality control procedures.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample.

Conversion factors:	$\mu\text{mol/L} \times 0.0585 = \text{mg/dL}$
	$\text{mg/dL} \times 10 = \text{mg/L}$
	$\text{mg/dL} \times 17.1 = \mu\text{mol/L}$

Limitations - interference

Criterion: Recovery within $\pm 3.4 \mu\text{mol/L}$ (0.199 mg/dL) of initial values of samples $\leq 34 \mu\text{mol/L}$ (1.99 mg/dL) and $\pm 10 \%$ of samples $> 34 \mu\text{mol/L}$.

Hemolysis:⁵ No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 $\mu\text{mol/L}$ or 800 mg/dL).

Criterion: Recovery within $\pm 1.7 \mu\text{mol/L}$ (0.099 mg/dL) of initial values of samples $\leq 17 \mu\text{mol/L}$ (0.995 mg/dL) and $\pm 10 \%$ of samples $> 17 \mu\text{mol/L}$.

Hemolysis in neonates:⁵ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 $\mu\text{mol/L}$ or 1000 mg/dL).

Lipemia (Intralipid):⁵ No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{6,7}

Indican: No significant interference from indican up to levels of 0.12 mmol/L or 3 mg/dL.

Cyanokit (Hydroxocobalamin) may cause falsely low results.

Results from certain multiple myeloma patients may show a positive bias in recovery. Not all multiple myeloma patients show the bias and the severity of the bias may vary between patients.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.⁸

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In certain cases specimens may give a direct bilirubin result slightly greater than the total bilirubin result. This is observed in patient samples when nearly all the reacting bilirubin is in the direct form. In such cases the result for the total bilirubin should be reported for both D-bilirubin and total bilirubin values.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi analyzers. Refer to the latest version of the carry-over evasion lists and the operator's manual for further instructions. US users refer to the Special Wash Programming document, available at usdiagnostics.roche.com, and the operator's manual for special wash instructions.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges**Measuring range**

Roche/Hitachi 902/MODULAR P analyzers
2.5-650 $\mu\text{mol/L}$ (0.146-38.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2. On instruments without rerun function, manually dilute samples having higher concentrations with 0.9 % NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate factor (e.g. 2).

Roche/Hitachi MODULAR D analyzer
2.5-550 $\mu\text{mol/L}$ (0.146-32.2 mg/dL)

Determine samples having higher concentrations via the rerun function. For samples with higher concentrations, the rerun function decreases the sample volume by a factor of 1.5. The results are automatically multiplied by this factor.

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

LoB = 1.7 $\mu\text{mol/L}$ (0.099 mg/dL)

LoD = 2.5 $\mu\text{mol/L}$ (0.146 mg/dL)

LoQ = 2.5 $\mu\text{mol/L}$ (0.146 mg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from $n \geq 60$ measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

