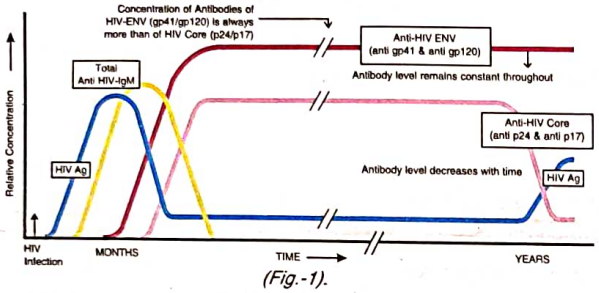
HIV TRI-DOT

First Method

Rapid Visual Test for the Qualitative Detection of Antibodies to HIV-1 & HIV-2 in Human Serum/Plasma
Separate Dots for HIV-1, HIV-2 & Control

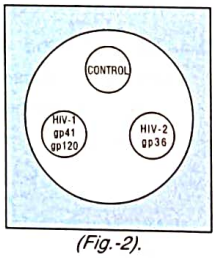
I. HISTORICAL REVIEW AND AETIOLOGY OF AIDS (Acquired Immuno Deficiency Syndrome)

First confirmed case of AIDS was identified in 1983 and by 1984 the etiologic agent, the Human Immunodeficiency Virus (HIV), subsequently named HIV-1 was isolated. Shortly afterwards in 1985 another retrovirus subsequently named HIV-2 was isolated in Africa. These two viruses belong to the retrovirus group and are slow viruses. The structure, gene organisation and serological behaviour of HIV-1 & HIV-2 and their complete nucleotide sequence has been determined. This knowledge has laid a foundation for the development of a new assay based on Recombinant DNA technology leading to the differential detection of antibodies to HIV-1 & HIV-2 (if present) in Human Serum or Plasma. Research has shown that antibodies produced against envelope gene are found in infected people as shown in graph, (Fig.-1).



(Fig.-1).

HIV TRI-DOT has been developed and designed using gp41, C terminal of gp120 & gp36 representing the immunodominant regions of HIV-1 & HIV-2 envelope gene structure respectively. The device (an immunofiltration membrane) includes a "Built-in Quality Control DOT" which will develop colour during the test, thereby, confirming proper functioning of the device, reagents and correct procedural application. This CONTROL DOT is the "Built-in Quality Control." (Fig.2)



(Fig.-2).

HIV TRI-DOT has been specially researched, developed and engineered using several thousands of serum/plasma specimens. It has also been evaluated by UNAIDS (WHO) Geneva, using samples of European, Asian, Latin American & African origin. The Sensitivity and Specificity has been extremely high in these samples of diverse origin.

The panel used for evaluation of HIV TRI-DOT by Institute of Tropical Medicine, WHO Collaborating Centre in AIDS, Belgium also included HIV-O Virus, which was found reactive with HIV TRI-DOT.

2. INTENDED USE

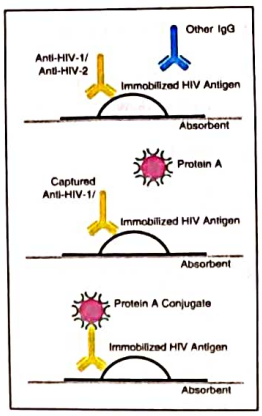
The HIV TRI-DOT Test is a visual, rapid, sensitive and accurate immunoassay for the differential detection of HIV-1 & HIV-2 antibodies (IgG) in Human Serum or Plasma using HIV-1 & HIV-2 Antigens immobilized on an immunofiltration membrane. The test is a screening test for anti-HIV-1 & anti-HIV-2 and is for *in vitro* diagnostic use only.

3. PRINCIPLE OF THE TEST

HIV antigens are immobilized on a porous immunofiltration membrane. Sample and reagents pass through the membrane and are absorbed into the underlying absorbent.

As the patient's sample passes through the membrane, HIV antibodies, if present, bind to the immobilized antigens.

Conjugate binds to the Fc portion of the HIV antibodies to give distinct pinkish purple DOT(s) against a white background. (Fig.-3)



(Fig.-3).

4. KIT DESCRIPTION

COMPONENTS	CONTENTS	PREPARATION
1. HIV TRI-DOT Test Device	Packed individually. Device has membrane with 1 Control & 2 Test Dots, one each for HIV-1 & HIV-2.	Cut open the pouch before use.
2. Buffer Solution	Buffer containing BSA and sodium azide.	Ready to use.
3. Protein-A Conjugate	Protein-A Conjugate in liquid form containing sodium azide.	Ready to use.
4. Sample Dropper	Long Plastic dropper provided for adding the sample.	

Store the kit at 2-8°C in the driest area available.
Bring all reagents and test components to room temperature (20-30°C) before use. Return entire kit at 2-8°C when not in use. DO NOT FREEZE TEST COMPONENTS.

5. MATERIAL REQUIRED BUT NOT PROVIDED

The kit contains all the items required to perform this test. But if the sample is viscous/turbid/contains particulate matter, a centrifuge will be required, to separate off the suspended matter. Since the test is completed in less than 5 minutes a timer or stop watch is not essential.

6. STORAGE

Store the entire kit at 2-8°C in the coolest and driest area available. The components are stable for 15 months from the date of manufacturing, when stored at 2-8°C. Do not use the kit beyond the expiry date. DO NOT FREEZE THE KIT COMPONENTS.

7. KIT PRESENTATION

50 Test Pack 100 Test Pack
200 Test Pack

8. WARNING FOR USERS

1. The use of disposable gloves is STRONGLY RECOMMENDED during the test.
2. In case there is a wound or cut in the hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. This Kit is for *in vitro* diagnostic use only.
5. All the samples to be tested should be handled as though capable of transmitting infection.
6. Spills should be decontaminated promptly with disinfectant.
7. Dispose of all specimens and materials used to perform the test appropriately using disinfectant.
8. The Protein-A Conjugate and Buffer Solution contain Sodium Azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amount of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to Remove Azide Salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).
9. Thoroughly wash hands with soap after the use of this kit. In case of a needle prick or other skin puncture or wounds, wash the hands with excess of water and soap.

9. PRECAUTIONS

1. Do not use kit components beyond the expiration date, which is printed on the kit.
2. Do not combine reagents from different batches during the same series, as they are optimized for individual batch to give best result.
3. Due to interchange of caps of the vials, the reagents may get contaminated. Care should be taken while handling the reagent caps to avoid cross contamination of the reagents. Place white nozzle cap on Buffer Solution vial and red cap on Protein-A Conjugate Vial after use.
4. Use a separate sample dropper for each sample and then discard it as biohazardous waste.
5. Avoid several times freezing and thawing of the sample to be tested.
6. Always allow each reagent to fall freely from the dropper tip. Do not touch the dropper tip to any surface; this may contaminate the reagent.
7. Avoid microbial and cross contamination of reagents.

10. SPECIMEN/SAMPLE COLLECTION & STORAGE

Collect blood in a clean dry sterile vial and allow to clot or separate the serum by centrifugation at room temperature. It is recommended that fresh sample should be used if possible. If serum is not to be assayed immediately it should be stored at 2-8°C or frozen at minus 20°C (-20°C). Only human serum or plasma should be used for the test. Haemolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

11. SPECIMEN/SAMPLE PROCESSING

(A) FROZEN SAMPLE:

The HIV TRI-DOT Test is best when used with fresh samples that have not been frozen and thawed. However, most frozen samples will perform well if the following suggested procedure is followed.

1. Allow the sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample can be centrifuged at 10,000 r.p.m. for 15 min.
2. Insert the dropper just below the top surface of the sample and withdraw one drop of sample. If the above procedure still yields a high background, dilute 1 drop of sample with 2 drops of normal saline. Use 1 drop of this diluted sample in the test.

(B) THICK OR VISCOUS SAMPLES:

Whenever possible, clear specimens should be used. However viscous, thick or turbid samples which may sometimes take more than 40-60 seconds to flow through the membrane should be centrifuged at 10,000 r.p.m. for 15 min. and retested on a fresh device to avoid inconsistent results.

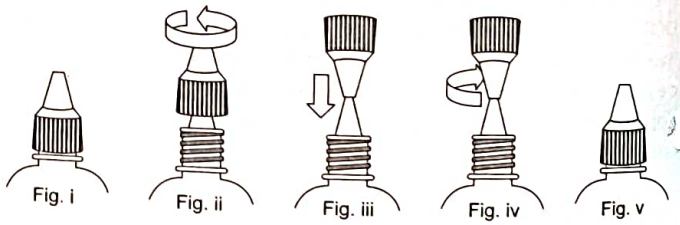
(C) TRANSPORTATION

If the specimen is to be transported it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

12. BEFORE YOU START

The Buffer Solution and Protein-A Conjugate vials are provided with closed nozzle and screw cap with pin(outside), then punture the nozzle before use as given below:

1. Before using reagents, keep the vial vertically straight and tap down gently on the working platform, so that reagents come down at the bottom of the vial.
2. To orifice the closed nozzle, press the inverted cap on the respective closed nozzle and give a half turn twist to ensure nozzle is properly orificed/ punctured as illustrated below in Fig. iii & iv:



13. ASSAY PROCEDURE

Take care of the following points before starting the test.

1. Bring all the reagents and specimens to room temperature (20°C-30°C) before beginning the test. The immunological sequence of reactions which take place during different procedural steps shows best performance at room temperature. DO NOT HEAT OR REPEATEDLY FREEZE/THAW SPECIMEN.

2. Place the required number of HIV TRI-DOT test devices at the working area.
3. Tear off the pouch and take out the device for performing the test. Write the sample number to be tested on the device.

4. While adding sample/reagents to the device, be sure to ALLOW EACH SOLUTION TO SOAK IN BEFORE ADDING THE NEXT SOLUTION.
   However drops of each solution should be added in continuous stream to wet the entire area of membrane.
5. If the solution does not soak-in within 40-60 seconds; observe the sample for any suspended particulate matter. If it is present, centrifuge the sample at 10,000 r.p.m. for 15 min. and use a fresh device to re-run the test. Refer to "SPECIMEN / SAMPLE PROCESSING".

6. All solutions and sample should be added to the CENTRE OF MEMBRANE.

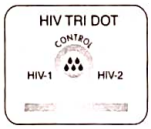
7. For consistent results, ensure FREE FALLING OF DROPS on the membrane.
8. Do not use kit components beyond the expiration date.
9. The liquid conjugate should not be subjected to frequent temperature fluctuations.
10. The procedural sequence of reagent addition should be strictly adhered to avoid any discrepant results.

14. TEST PROCEDURE

1. Add 3 drops of Buffer Solution to the centre of the device

2. Hold the dropper vertically and add 1 drop of patient's sample (serum or plasma) using the sample dropper provided (use a separate sample dropper for each specimen to be tested).

3. Add 5 drops of Buffer Solution.



4. Add 2 drops of Protein-A Conjugate directly from the conjugate vial.

5. Add 5 drops of Buffer Solution and read results.

Read results immediately and discard the device considering it to be potentially infectious.

IMPORTANT: *IT IS IMPORTANT TO ALLOW EACH SOLUTION TO SOAK IN THE TEST DEVICE BEFORE ADDING THE NEXT SOLUTION.*

15. INTERPRETATION OF RESULTS

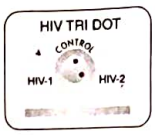
NON-REACTIVE

1. If only One DOT (only the Control Dot) appears as shown in fig., the specimen is non reactive for antibodies either to HIV-1 or HIV-2. Interpret sample as non-reactive.

REACTIVE

1. If two DOTS, one for the control and the other for HIV-1 appear as shown in Fig., the specimen is reactive for antibodies to HIV-1.

2. If two DOTS, one for the control and the other for HIV-2 appear as shown in Fig., the specimen is reactive for antibodies to HIV-2.



3. If all the three DOTS, one each for control, HIV-1 & HIV-2 appear as shown in Fig., the specimen is reactive for antibodies to HIV-1 & HIV-2.

INVALID TEST

If no DOT appears after the test is complete, either with clear background or with complete pinkish/purple background the test indicates ERROR. This may indicate a procedural error or deterioration of specimen/reagens or particulate matter in the specimen. The specimen should be tested on a new device.

(If the problem persists, please call our Technical/ Customer service cell, Parwanoo, Himachal Pradesh, Phone: 01792-232253).

IMPORTANT

1. All initially reactive samples should be subjected to centrifugation at 10,000 r.p.m. for 15 min. It is recommended that this centrifugation step should be carried out prior to sending the sample for the Western Blot. The test should be repeated with supernatant collected after centrifugation. If no dot appears on repetition, it indicates a falsely reactive sample. A truly reactive dot will not show much change in its colour intensity after centrifugation. The false reactivity of the sample is generally due to the presence of suspended particulate matter in the serum which may or may not be visible to the naked eye.
   This critical step of centrifuging a reactive sample should be faithfully followed. Its correct application makes the test EXTREMELY SENSITIVE and completely eliminates the possibility of false reactivity.
2. Sometimes, if the sample solution does not soak-in within 40-60 seconds, the sample should be observed for any suspended particulate matter. If it is present, centrifuge the sample at 10,000 r.p.m. for 15 min. Use a fresh device to re-run the test.
3. Test dots HIV-1 and HIV-2 either dark or light in pink colour should be considered reactive.
4. ***Sample found to be reactive by the above screening test must be confirmed by standard supplemental assay, like Western Blot.***

16. LIMITATIONS OF THE TEST

1. The kit works best when used with fresh samples. Samples which have been frozen and thawed several times contain particulates which can block the membrane, hence resulting in improper flow of reagents and high background colour which may make the interpretation of results difficult.
2. Optimum test performance depends on strict adherence to the test procedure as described in this manual.
   Any deviation from test procedure may lead to erratic results.
3. HIV-1 and HIV-2 viruses share many morphological and biological characteristics. It is likely that due to this, their antibodies have a cross reactivity of 30-70%. Appearance of dots for HIV-1 and HIV-2 antibodies on the test device does not necessarily imply co-infection from HIV-1 & HIV-2.
4. Some samples show cross reactivity for HIV antibodies. **Following factors are found to cause false positive HIV antibody test results:** Naturally occurring antibodies, Passive immunization,

Leprosy, Renal Disorders, Tuberculosis, Myco-bacterium avium, Herpes simplex, Hypergamma-globulinemia, Malignant neoplasms, Rheumatoid arthritis, Tetanus vaccination, Autoimmune diseases, Blood Transfusion, Multiple myeloma, Haemophelia, Heat treated specimens, Lipemic serum, Anti-nuclear antibodies, T-cell leukocyte antigen antibodies, Epstein Barr virus, HLA antibodies and other retroviruses.

5. **This is only a screening test. All samples detected reactive must be confirmed by using HIV Western Blot.** Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serological data, should be considered. The results should be reported only after complying with above procedure.

17. PERFORMANCE CHARACTERISTICS

Performance of the HIV TRI-DOT with reference to sensitivity and specificity has been evaluated in house with fresh as well as frozen samples from low risk as well as high risk groups by using a panel containing 1325 nos. of known serum/ plasma samples including cross reacting samples. The results of all the samples with a defined HIV status were fully comparable with those of HIV TRI -DOT. The results of the in-house study done are as follows:

No. of Samples	Status	HIV TRI-DOT	HIV TRI-DOT
		+ ve	- ve
50	ELISA +ve	50	-
1275	ELISA -ve	1	1274

Sensitivity: 100%

Specificity: 99.84%

Precision: Within-run and between-run precisions have been determined by testing 10 replicates of 10 samples: 7 HIV-1 positive (1 strong, 1 moderate & 5 weak), 1 HIV-2 positive and 2 HIV negative. The C.V.(%) of all the samples were within 10%.

18. DISPOSAL

Discard the test device immediately after reading result. Before discarding it, add few drops of disinfectant on device membrane and on all other items used for handling serum. Put all items to be disposed in Disposable Bags and dispose off accordingly.

19. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

20. REFERENCES

1. Ajaka Z.L. et.al. (1994). HIV-1/HIV-2 seronegativity in HIV-1 subtype O infected patients. The Lancet., 343, 1393-1394.
2. Aman E., Brosius J., (1985): "ATG Vectors" for Regulated High level Expression of Cloned Genes in *E. coli* Gene, 40, 183-190.
3. Dolt J., Sotz T., Schafer, T., Bergmann, C., (1968): Expression, Secretion and Processing of Hirudin in *E. coli*, using the Alkaline Phosphatase Signal Sequence. FEBS Letter, 202, 373-377.
4. Guyader, M, Emerman, M, Sonigo, P., Clavel. F, Montaginer L., Alizon M., (1987): Genome Organization and Transactivation of the Human Immunodeficiency Virus Type 2. Nature, 326, 662-669.
5. Kan N.C. Franchini G., Wong-Staal F., Dukbois, G.C., Robey. W.G., Lautenber, J.A., Papas T.S., (1986): Identification of HTLV-III/LAV SOR Gene Product and Detection of Antibodies in Human Sera. Science, 231, 1553-1555.
6. Schauder B., Blocker H., Frank. R; Mc Carthly. J.E.G., (1987): Inducible Expression of Cloned Genes in *E. coli* Gene., 40, 183-190.
7. Schumacher T.T., Garrett P.E., Tegtmeyer G.E., Thomas D., (1988): Comparative Detection of Anti-HIV in Early HIV seroconversion. J. Clinical Immunoasay, 11, 130-131.
8. Smith B.B., Johnson K.S., (1988): Single-Step Purification of Polypeptides W Expressed in *Escherichia coli* fusion with Gluthathione S-Tranferase. Gene., 52, 279-283.
9. Gurtler L.G. et.al. (1994). A new subtype of Human Immunodeficiency virus type 1 (MVP-1580) from conversion. J. of Virology., 68, 1581-1585.

R-05

For *in vitro* diagnostic use only, not for medicinal use

Manufactured & Marketed By:

DIAGNOSTIC ENTERPRISES

Plot No.: 26, Indl. Estate, Sector-1, Parwanoo - 173 220, (H.P.)

Phone: 01792-232253 E-mail: de@diagnosticenterprises.com

MANTD001

Size : 137 x 218 mm

Second Method

Combiquic®

HIV & HCV

Rapid immunoconcentration assay for detection of antibodies to HIV-1 & 2 and HCV in human serum/plasma

DEVICE

INTENDED USE

COMBIQUIC® is rapid, qualitative, immunoconcentration (flow through) assay for the simultaneous and differential detection of antibodies to HIV1&2 and HCV in human serum/plasma. For Professional use.

SUMMARY

HIV

Acquired Immuno Deficiency Syndrome (AIDS) is caused by at least two retroviruses, the HIV-1 and the HIV-2, collectively referred to as HIV-1/2. Antibodies to HIV-1 envelope protein (gp120), transmembrane protein (gp 41) and HIV-2 transmembrane protein (gp36) are prevalent in sera of individuals with AIDS or AIDS related complex (ARC) or who are at high risk of contracting AIDS. Detection of these antibodies indicates exposure to the HIV-1/2 virus.

HCV

HCV is as single-stranded RNA virus containing a linear genome with a length of about 9,600 nucleotides with positive polarity. It is now recognized that HCV infection is the major ethiological agent of post transfusion hepatitis type non-A, non-B, HCV infection frequently progresses to chronic liver disease. On the basis of phylogenetic analysis. HCV has been grouped into six major genotypes, each of which contains one or more subtypes. The distribution of HCV genotypes varies in different geographical areas.

For the detection of HIV, synthetic peptides representing the highly immunodominant regions of HIV-1 &2 are coated on the membrane of **COMBIQUIC®**. Combination of these peptides in a new generation assay format (advanced flow-through) affords specific and early detection of seroconversion following exposure to HIV.

For the detection of HCV, **COMBIQUIC®** employs recombinant protein derived from Core, NS3, NS4 and NS5 regions of the HCV genome. For its HCV- detection module, **COMBIQUIC®** is a third generation assay the uses a cocktail of recombinant antigens derived from multiple HCV genotypes.

PRINCIPLE

COMBIQUIC® utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. It comprises of a test device striped with distinct bands or purified gp120, gp41 and gp36 synthetic peptides specific to HIV at a test region 'HIV' and recombinant antigens derived from the Core, NS3, NS4 and NS5 regions of the HCV genome (from multiple HCV-genotypes) specific to HCV at test region 'HCV'. The third band striped at region 'CNT' corresponds to the assay performance control and contains purified Protein -A . At first the membrane assembly is hydrated with wash buffer and then the specimen is added. Antibodies to HIV and /or HCV, if present, are captured by the respective antigens. After washing with wash buffer. Protein-A conjugates gold sole reagent is added to reveal the presence/absence of bound antibodies. Post final wash, a positive reaction is visualized by the appearance of purple colored bands at the test regions. The absence of bands at the test regions is a negative test result . The appearance of control band serves to validate device functionally, sample addition, reagent and assay performance.

KIT COMPONENTS

Each kit of COMBIQUIC® contains the following component

Device DEVICE	Stripped with HIV-1&2, HCV specific antigens and procedural control, individually pouched with a desiccant
Disposable sample applicator PIPETTE	Inside each individual pouch, for single use only
Dropper bottle for wash buffer BUF	Buffer containing surfactant and preservatives, ready to use
Dropper bottle for conjugate CON	Protein-A conjugated to colloidal gold in a stabilizing solution, ready to use
Package Insert	One package insert provided in each kit

REF	402080050
Σ	50 Tests

STORAGE AND STABILITY

COMBIQUIC® should be stored between 2-8°C for the duration of shelf life as indicated on the outer carton. Once the pouch is opened, the device must be used immediately.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Disinfectant.
2. Disposable gloves.
3. Biohazard waste containers.

Second Method

SAMPLE COLLECTION

1. **COMBIQUIC®** uses human serum/plasma as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. Preferably use fresh sample. However, specimen may be stored refrigerated (2- 8 °C) for short duration. For long storage, freeze at – 20 °C or below.
4. If serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum.
5. Repeated freezing and thawing of the specimen should be avoided.
6. Do not heat inactivate before use.
7. Do not use turbid, lipaemic and hemolysed serum/plasma.
8. Do not use hemolysed, clotted or contaminated specimens.
9. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
10. Refrigerated specimens must be brought to room temperature prior to testing.

PRECAUTIONS

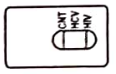
1. For in vitro diagnostic use only. NOT FOR MEDICINAL USE.
2. Bring all reagents and specimen to room temperature before use.
3. Do not use beyond expiration date.
4. Read the instructions carefully before performing the test.
5. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
6. Handle all specimens as if potentially infectious.
7. Do not pipette any material by mouth.
8. Do not eat, drink or smoke in the area where testing is done.
9. Use protective clothing and wear gloves when handling samples.
10. Use absorbent sheet to cover the working area.
11. Immediately clean up any spills with sodium hypochlorite.
12. Dispose off all the reagents and material used as if they contain infectious agent.
13. Do not mix components of one lot with another.
14. If desiccant color at the point of opening the pouch has turned from blue to white, another test device must be run.

TEST PROCEDURE

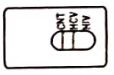
1. Bring the sealed aluminium foil pouch of **COMBIQUIC®** device to room temperature.
2. Open a foil pouch by tearing along the "notch".
3. Remove the testing device and the sample applicator. Once opened, the device must be used immediately.
4. Label the device with specimen identity.
5. Place the testing device on a flat horizontal surface.
6. Holding the dropper vertically, add **two drops** of wash buffer in the reaction well and allow to soak completely.
7. Add **one drop** (25µl) of serum specimen and allow to pass through.
8. Add **three drops** of wash buffer and allow to soak completely.
9. Add **two drops** of Protein-A gold conjugate and allow to pass through.
10. Add **two drops** of wash buffer and allow to pass through.
11. Record the results immediately.

RUN CRITERIA

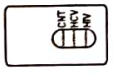
A purple colored band must appear in the control area marked 'CNT'. If control band does not appear the test is invalid. Absence of control band indicates either deterioration of the kit or absence of sample addition. Repeat test, making sure that sample has been added.



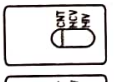
HIV-1 and/or HIV-2 Positive
A colored band appears in the Control area marked 'CNT' as well as in the area marked 'HIV'. The sample reactive for HIV1 and/or HIV2.



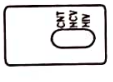
HCV Positive
A colored band appears in the Control area marked 'CNT' as well as in the area marked HCV'. The sample reactive for HCV.



HIV-1 and/or HIV-2 and HCV Positive (Co-infection)
A colored band appears in the Control area marked 'CNT' as well as in the area marked 'HIV'& 'HCV'. The sample reactive for HIV1 and/or HIV2 and HCV.



Negative
Only one colored band appears in the Control area marked 'CNT'.



Invalid
The test should be considered invalid if the control band 'CNT' does not appear. The test is also invalid if only the test band and no control band appears. Repeat the test with a new **COMBIQUIC®** HIV/HCV device.

PERFORMANCE CHARACTERISTICS

In an in-house study, the performance of **COMBIQUIC®** device was evaluated using a panel of specimens of positive (at varying stages of seroconversion) for HIV1 & 2 and HCV along with negative sera in comparison with commercially available ELISA kits.

SAMPLES TESTED	COMBIQUIC®		Licensed ELISA	
	HIV1&2	HCV	HIV1&2	HCV
Total No. of Samples tested	1228	569	1228	569
Total No. of Negatives	1064	517	1064	519
No. of HIV positive	164	-	164	-
No. of HCV positive	-	50	-	50

Based on the above evaluation, the sensitivity and specificity of **COMBIQUIC®** is as follows

PARAMETER	HIV1&2	HCV
Sensitivity	100%	100%
Specificity	100%	99.61%

EVALUATION WITH SEROCONVERSION PANEL : HIV MODULE

COMBIQUIC® was evaluated with anti -HIV-1 Seroconversion Panel D (PRB904) obtained from Boston Biomedica Inc., USA. The results were found to be satisfactory and are as follows:

Panel ID#	Days Since 1st bleed	ORGANON TEK. HIV*	DUPONT Western Blot	Roche RNA PCR	COMBIQUIC®
PRB 904-01	0	0.5	No Bands	BLD**	Negative
PRB 904-02	21	0.4	No Bands	BLD	Negative
PRB 904-03	49	0.5	No Bands	Positive	Negative
PRB 904-04	92	5.1	18, 24, f41, 55, f65, 120, 160	Positive	HIV-1Positive
PRB 904-05	99	4.9	18, 24, 41, 51, 55, 65, 120, 160	Positive	HIV-1 Positive

** Below Detection Limit

EVALUATION WITH SEROCONVERSION: HCV MODULE

By using Seroconversion panel from Boston Biomedica Inc., USA (Panel ID: PHV 901), that contains11 samples, the sensitivity of **COMBIQUIC®** was evaluated. The results were found to be satisfactory and are as follows:

Panel ID#	Days since first bleed	Abbott HCV 3.0	Ortho HCV 3.0	Ortho RIBA 3.0	COMBIQUIC®
PHV 901-01	0	0.2	0.0	NEGATIVE	NEGATIVE
PHV 901-02	72	0.2	0.0	NEGATIVE	NEGATIVE
PHV 901-03	104	1.0	5.9	POSITIVE	POSITIVE
PHV 901-04	106	1.0	6.0	POSITIVE	POSITIVE
PHV 901-05	111	1.2	6.1	POSITIVE	POSITIVE
PHV 901-06	113	1.3	6.0	POSITIVE	POSITIVE
PHV 901-07	138	9.0	> 9.1	POSITIVE	POSITIVE
PHV 901-08	146	6.8	7.4	POSITIVE	POSITIVE
PHV 901-09	166	> 10.6	> 9.1	POSITIVE	POSITIVE
PHV 901-10	173	> 10.6	9.1	POSITIVE	POSITIVE
PHV 901-11	209	> 10.6	> 9.1	POSITIVE	POSITIVE

Data other than that of ***COMBIQUIC®*** *is supplied by BBI, USA. Numerical values are expressed as cut-off ratios. Ratios more than or equal to 1.0 are considered positive.*

INTRA-ASSAY PRECISION STUDY

Two samples-One HCV-positive and the other HIV-positive was assayed 10 times on the same day.
Results: No variation in results was observed indicating 100 % correlation.

INTER-ASSAY PRECISION STUDY

Two samples-One HCV-positive and the other HIV-positive was assayed 3 times on 3 different days.
Results: No variation in results was observed indicating 100 % correlation.

LIMITATIONS OF THE STUDY

1. The addition of reagents must be accomplished without interruptions. After addition of the wash buffer, in step # 10 of the procedure. If background in the reaction port is high, the samples must be re-centrifuged @ 3000 rpm for 15 minutes so as to pellet invisible particulate matter. Subsequently, test should be re-run using clear supernatant with a fresh **COMBIQUIC®** device.

Size : 137 x 218 mm

2. Absence of antibodies to HIV or HCV does not indicate that an individual is absolutely free of HIV or HCV as the collection of samples and its timing vis-a-vis seroconversion will influence the test outcome.
3. Since various tests for HIV and HCV differ in their performance characteristics and antigenic composition, the reactivity patterns may differ.
4. Do not compare the intensity of the test lines and the control lines to judge the concentration of the antibodies in the test sample.
5. Testing of pooled specimens is not recommended.
6. Though **COMBIQUIC®** is an sensitive and reliable screening test, it should be used as a sole criterion for diagnosis of HIV/HCV infection.
7. All positive specimens should be further tested using appropriate supplemental/confirmatory tests.
8. As **COMBIQUIC®** is read by visual inspection of colored bands, reading of the test is subjective for specimens giving weak colored band(s).
9. A weak positive result may be due to cross reactivity or low or borderline titers.
10. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) Clinical Significance of Hepatitis C Virus Genotypes, Nizar N.Zein, Clinical Microbiology Reviews, Vol.13 No.2, 223-235, April 2000.(2) Perspectives for the Treatment of Infections with Flaviviridae, Pieter Leyssen, Eric De Clerq, John Neyts, Clinical Microbiology Reviews, Vol.13 No.1, 67-82, Jan.2000. (3) Principle and Practice of Infectious Diseases, Mandell, Burnett and Dolin, 5th Edn. Vol. 1 Part-II, 1307-1331, 2000, Churchill Livingstone Publications. (4) Diagnosis of Human Immunodeficiency Virus Type 1 Infection with Different Subtypes Using Rapid Tests, Susan Phillips, Timothy C.Granade, Chou-Pong Pau, Debra Candal, Dale J.Hu, Bharat S.Parekh, Clinical and Diagnostic Laboratory Immunology, Vol.7 No.4, 698-699, July 2000. (5) Principle and Practice of Infectious Diseases, Mandell, Bennett and Dolin, 5th Edn., Vol 1-Part II, 1332-1528, 2000,Churchill Livingstone Publications. (6) Current Concepts in Human Immunodeficiency Virus Infection and AIDS, Stanley. Schwartz, Madhavan P.N.Nair, Clinical and Diagnostic Laboratory Immunology, Vol.6 No.3, 295-305, May 1999. (7) Restricted Isotypic Antibody reactivity to Hepatitis C Virus Synthetic Peptides in Immunocompromised Patients, Marisol Deves Arlette De Saez, Graciela Leon, Firelei Sirit, Clarisa Coson, Henry Bermudez, Ferdinando Liprandi, Oscar Noya, Flor H.Pujol, Clinical and Diagnostic Laboratory Immunology, Vol.6 No.2, 279-281, May 1999. (8) A Field Test for the Detection of Antibodies to Human Immunodeficiency Virus Types 1 and 2 in Serum or Plasma, Anthony Burgess-Cassler, Gustavo Barriga Angulo, Stephen E.Wade, Patricia Castillo Torres, Willfried Schramm, Clinical and Diagnostic Laboratory Immunology, Vol.3 No.4, 480-482, 1996. (9) Immunodiagnosis of Viral Hepatitis A to E and Non-A to -E, Gang Yang, Girish N.Vyas, Clinical and Diagnostic Laboratory Immunology, Vol.3 No.3, 247-256, May 1996. (10) Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from patients with AIDS and at risk of AIDS Robert C.Gallo, Syed Z.Salahuddin, Mikulas Popovic, Gene M.Shearer, Mark Kaplan, Barton F.Haynes, Thomas J. Palker, Robert Redfield, James Oleske, Bijan Safai, Gilbert White, Paul Foster, Phillip D.Markham, Science, Vol. 224, 500-502, 4 May 1984. (11) Retroviruses (HTLV-III) in the serum of Patients with AIDS, M.G.Sarangadharan, Mikulas Popovic, Lillan Bruch, Jorg Schupbach, Robert C.Gallo, Science, Vol.224, 506-508, 4 May 1984. (12) Data on file. Qualpro Diagnostics.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	DEVICE — Device
Manufacturer	IVD — *In vitro* Diagnostic Medical Device	This side up	BUF — Wash Buffer
Use by	REF — Catalogue Number	Do not reuse	CON — Protein A Gold Conjugate
Contains sufficient for <n> tests	LOT — Batch Number / Lot Number	PIPETTE — Disposable Plastic Sample Applicator	

Manufactured by:

Qualpro Diagnostics

A Division of Tulip Diagnostics (P) Ltd.

88/89, Phase II C, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

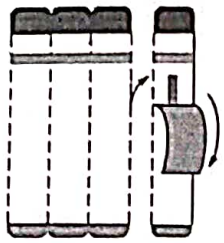
0717/VER-01

Alere Determine HIV-1/2

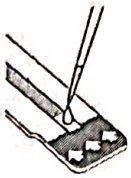
Third Method

PROCEDURE

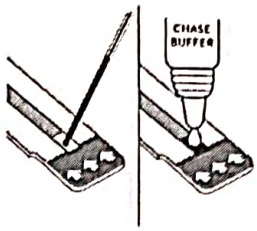
1 Prepare Test
Tear one strip from the right and remove cover.



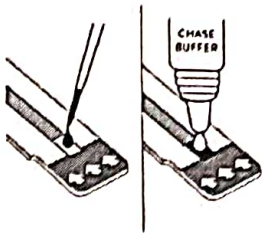
2 Add Sample
Plasma or Serum
Add 50µL of serum or plasma to the sample pad and wait 15 minutes



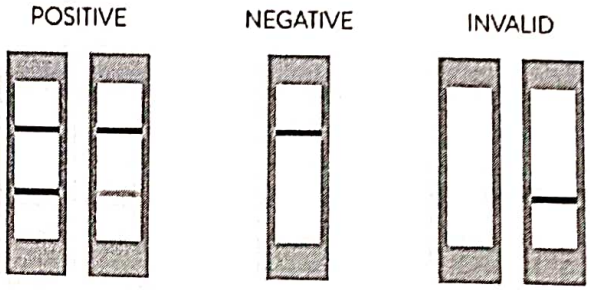
Fingerstick Whole Blood.
Add 50 µL of whole blood to the Sample Pad. When all the blood is absorbed into the Sample Pad, immediately apply one drop of Chase Buffer to the Sample Pad.



Venipuncture Whole Blood.
Add 50 µL of whole blood (precision pipette) to the Sample Pad. Wait 1 minute and add one drop of Chase Buffer.



3 Read Results
Read the results at 15 mins to 60 mins maximum



PRODUCT INFORMATION

INFORMATION TYPE	PRODUCT DETAIL
Method	Lateral flow
Time to results	15 mins.
Storage conditions	2 - 30°C
Shelf life	18 months
Sample type	Serum/Plasma/ Whole blood
Kit contents	20 or 100 test units, Package Insert

ORDERING INFORMATION

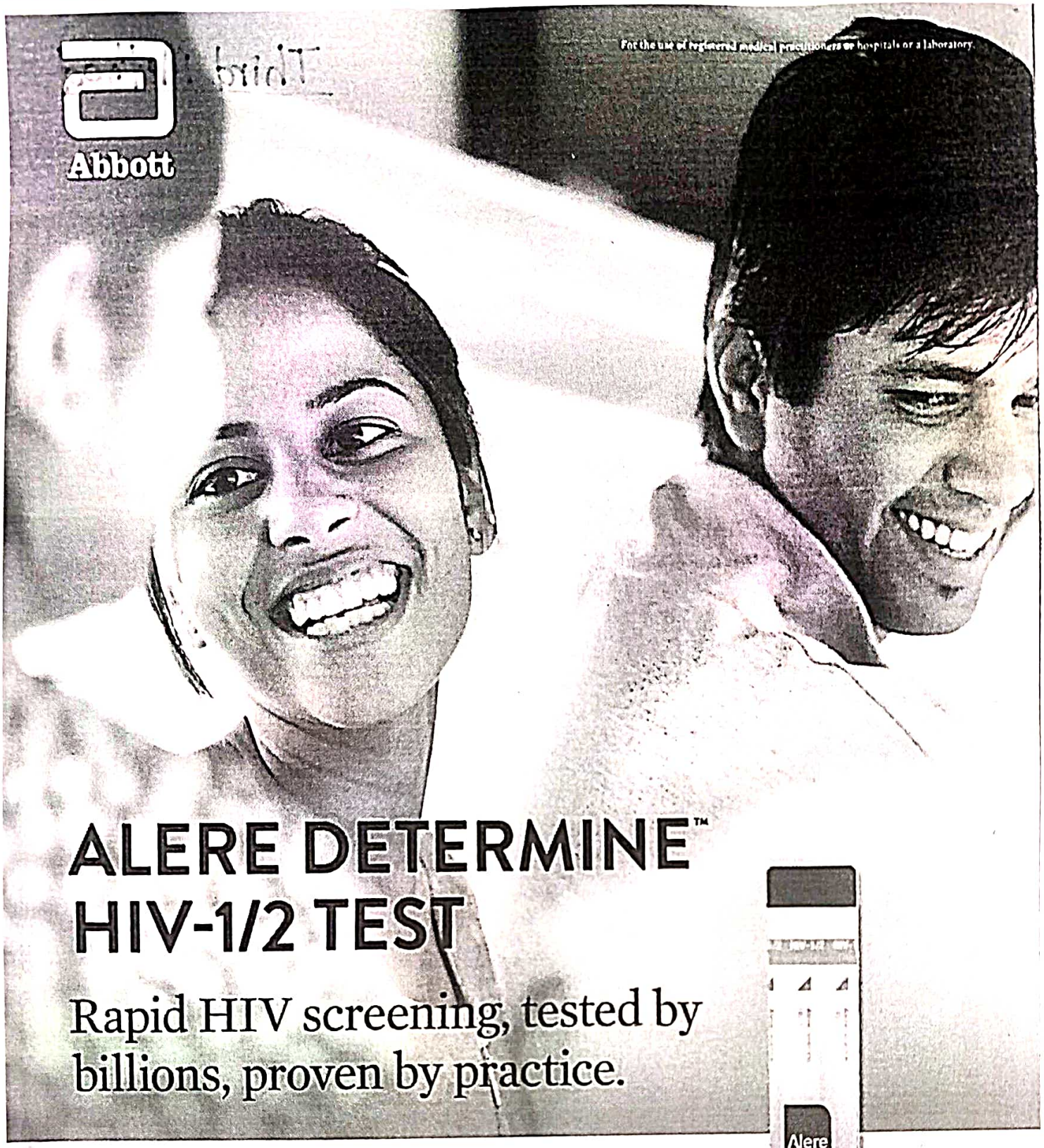
PRODUCT NAME	CE MARKED	NON CE MARKED
Alere Determine HIV-1/2 (x20)	7D2346	7D2342
Alere Determine HIV-1/2 (x100)	7D2347	7D2343
Chase Buffer*	7D2243	7D2243
EDTA-Capillary Tubes (x100)	7D2227	7D2222

*For whole blood, order Chase Buffer
Kindly refer to the Pack insert for complete Product information.

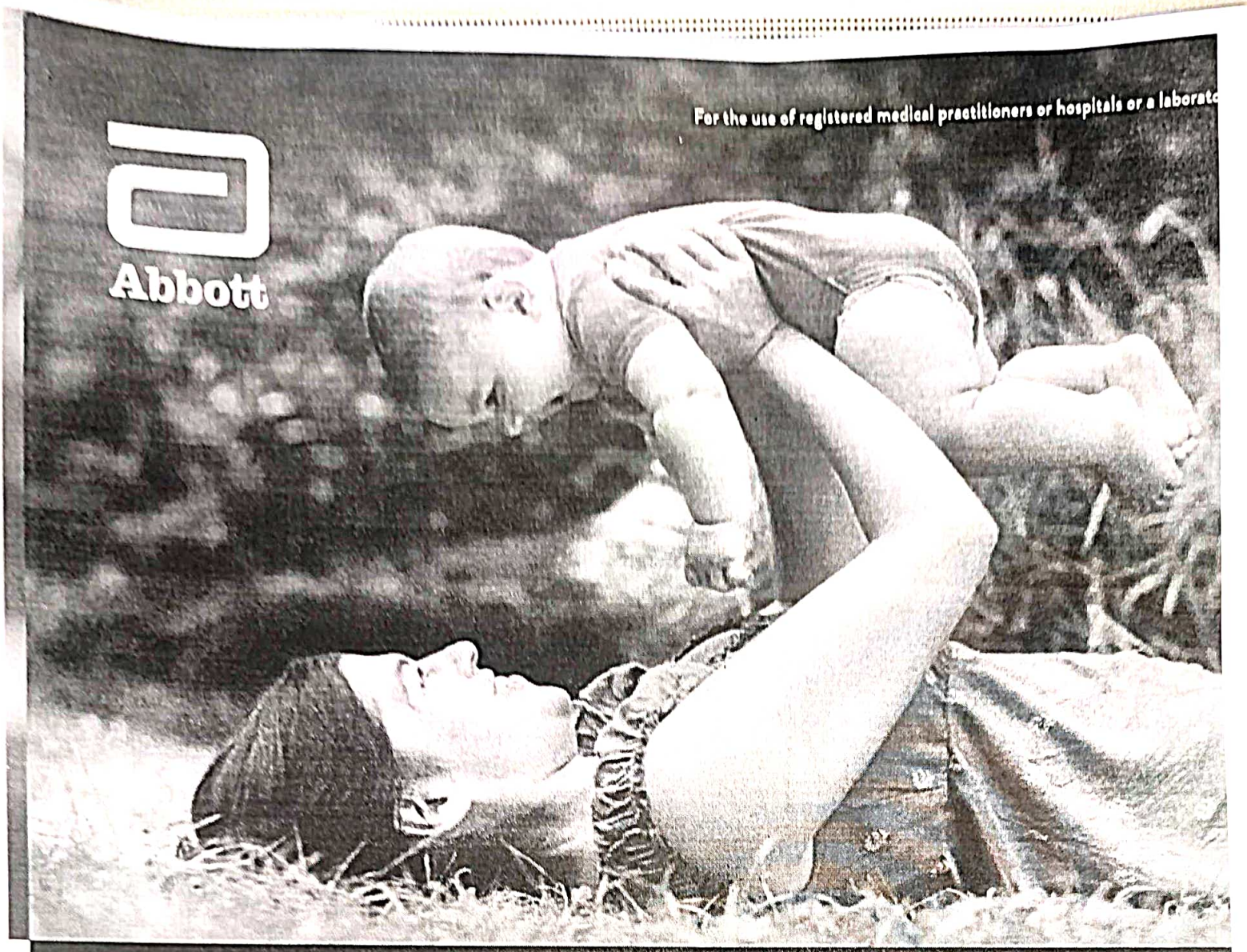
CONTACT YOUR LOCAL ABBOTT REPRESENTATIVE TODAY, OR VISIT ABBOTT.COM/POCT

Alere Medical Pvt. Ltd.
No 404, 4th Floor, BPTP Park Centra, NH-8,
Gurgaon – 122001 (Haryana) India
Phone: +91 124 4569000
Fax: +91 124 4569011

Abbott



Alere Determine HIV-1/2 detects all known subtypes of HIV. This versatility helps healthcare workers across the world diagnose individual infection, monitor HIV prevalence and enable fast linkage to care.



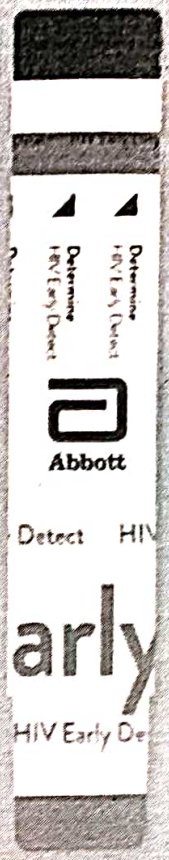
MAKE EVERY TESTING OPPORTUNITY COUNT

DETERMINE™ HIV EARLY DETECT

The New Standard in HIV Point-of-Care Testing

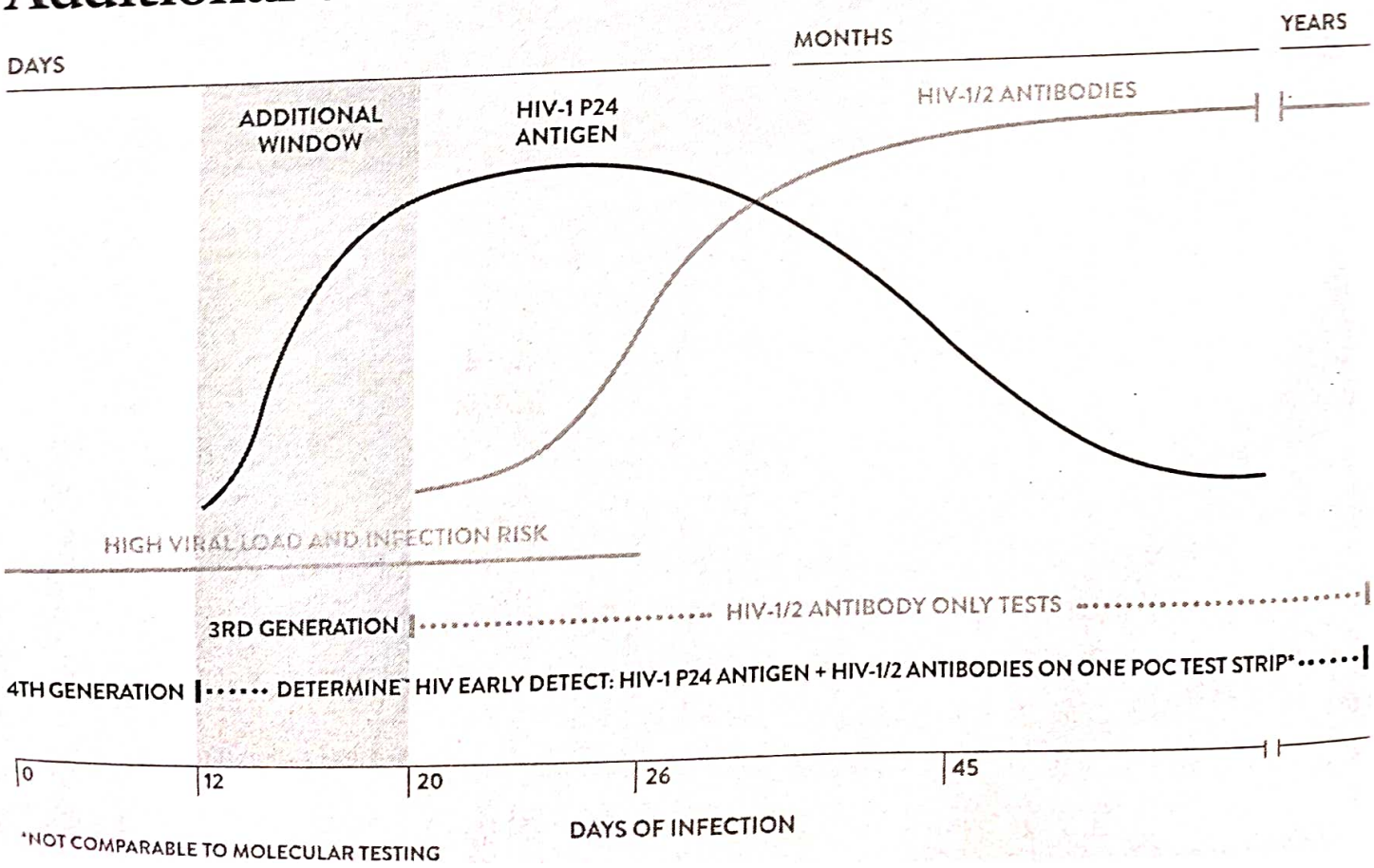
4th Generation Technology: HIV-1 p24 Antigen Plus HIV-1/2 Antibodies on a Single Test

Determine™ HIV Early Detect is an *in vitro*, visually read, qualitative immunoassay for the detection of antibodies (Ab) to HIV-1 and HIV-2 and the detection of non-immunocomplexed (free) HIV-1 p24 antigen (Ag) in human capillary and venous whole blood, plasma or serum.



It detects more acute infections compared to 2nd and 3rd generation tests, which helps close the window period and enables increased case finding at a time when individuals are highly infectious.

Additional Window Period Increases Case Finding



A pathway to improved prognosis with 4th generation POC testing

IMPROVED ACCESS
More individuals are empowered to know their status through point-of-care testing.

EARLIER DETECTION
Leading to increased case finding of highly infectious individuals.

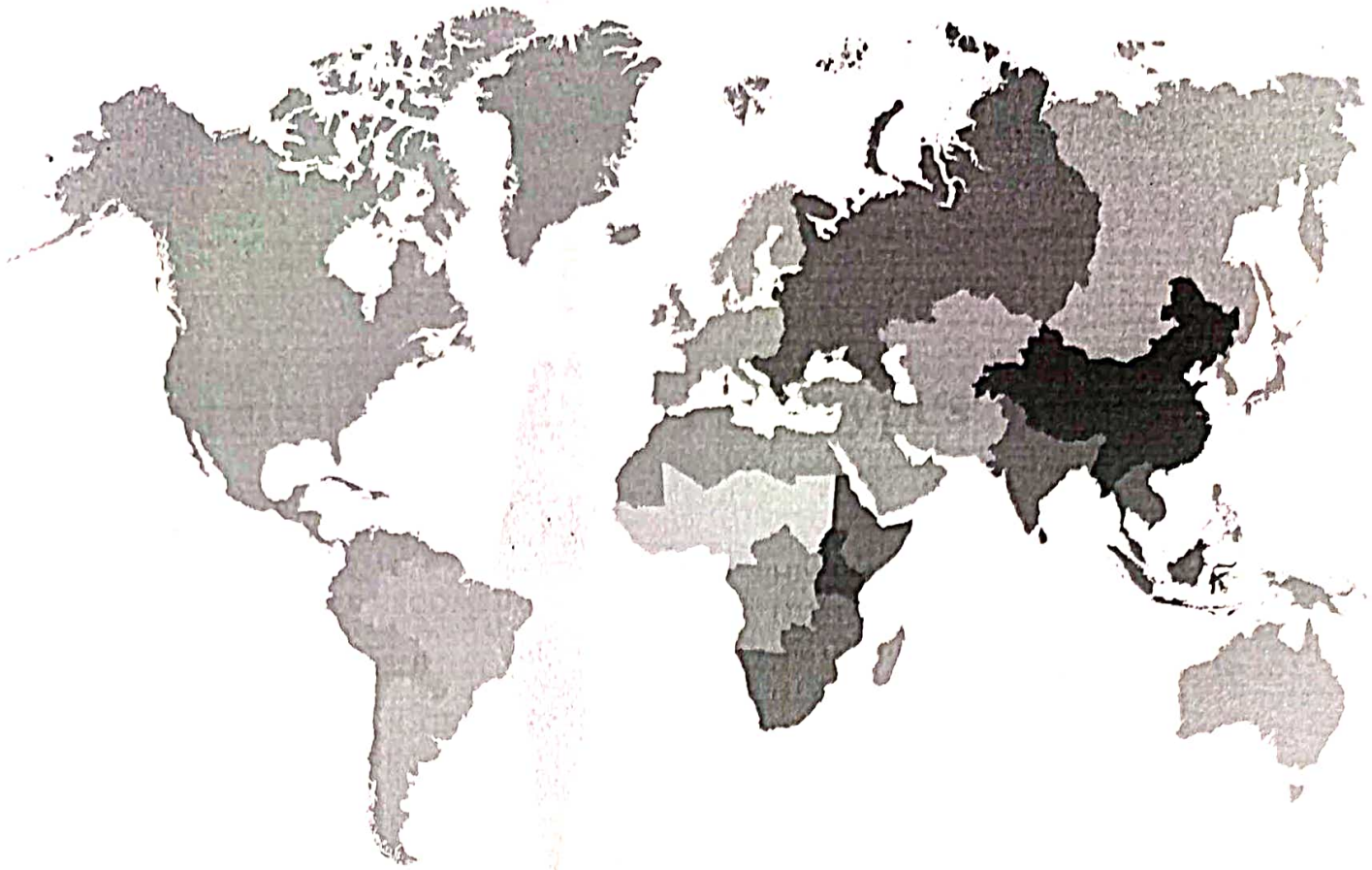
RAPID, ACCURATE SCREENING
Helps close the window period and provides confidence in results at the point of care.

IMMEDIATE COUNSELLING
Reduces patient loss to follow-up and allows immediate partner notification.

FASTER ASSESSMENT AND STAGIN
Provides fast linkage to care and decreases risk of onward transmission

TIMELY PATIENT MANAGEMENT
Ability to commence appropriate treatment sooner.

IMPROVED PROGNOSIS
Detecting HIV earlier leads to better patient outcomes.



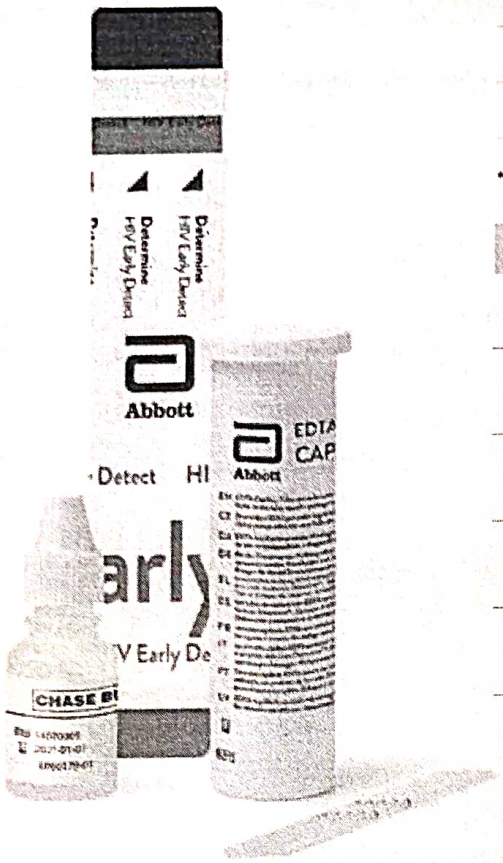
This map shows the global distribution and genetic diversity of the nine major subtypes, known as "clades," and recombinants of
Reprinted with permission from http://www.iavireport.org, published by the International AIDS Vaccine Ini

Distribution of HIV-1 Subtypes[1]

- B
- CRF02_AG, OTHER RECOMBINANTS
- CRF01_AE, B
- F, G, H, J, K, CRF01 OTHER RECOMBINANTS
- A, B, AB RECOMBINANT
- B, C, BC RECOMBINANT
- B, F RECOMBINANT
- A
- C
- D
- INSUFFICIENT DATA

HIV-1 ANTIGENS
HIV-1 group M subtype A, B, C, D, F, G, H and CRF01-AE, CRF02-AG, and HIV-1 group O are all detectable with Determine™ HIV Early Detect[2]

HIV ANTIBODIES
HIV-1 group M subtype A, B, C, D, F, G, H, J, K, CRF01-AE, CRF02-AG, CRF03-AB CRF05-DF, CRF09-A/U, CRF11-cpx, HIV-1 group O and HIV-2 are all detectable with Determine™ HIV Early Detect[2]



Performance

Sensitivity**	100%
Specificity	99.72%

**Please refer to the Product Insert for the analytical sensitivity of HIV-1 p24 Antigen.

PRODUCT INFORMATION	
Method	Lateral flow
Time to results	20 minutes
Storage conditions	2°C–30°C
Sample volume	50 µL
Test shelf life	18 months
Sample types	Serum/plasma, fingerstick whole blood and venipuncture whole blood

New Possibilities in Patient Care

By closing the window period, Determine™ HIV Early Detect sets a new standard in POC testing. This simple test delivers fast, accurate results in 20 minutes, introducing new possibilities in patient care.

ORDER INFORMATION

PRODUCT	CATALOGUE NUMBER
Determine™ HIV Early Detect 20 Test Kit	7D2842
Determine™ HIV Early Detect 100 Test Kit	7D2843
Chase Buffer (for 100 tests)	7D2243
EDTA Capillary Tubes (100)	7D2222

Make Every Testin Opportunity Count

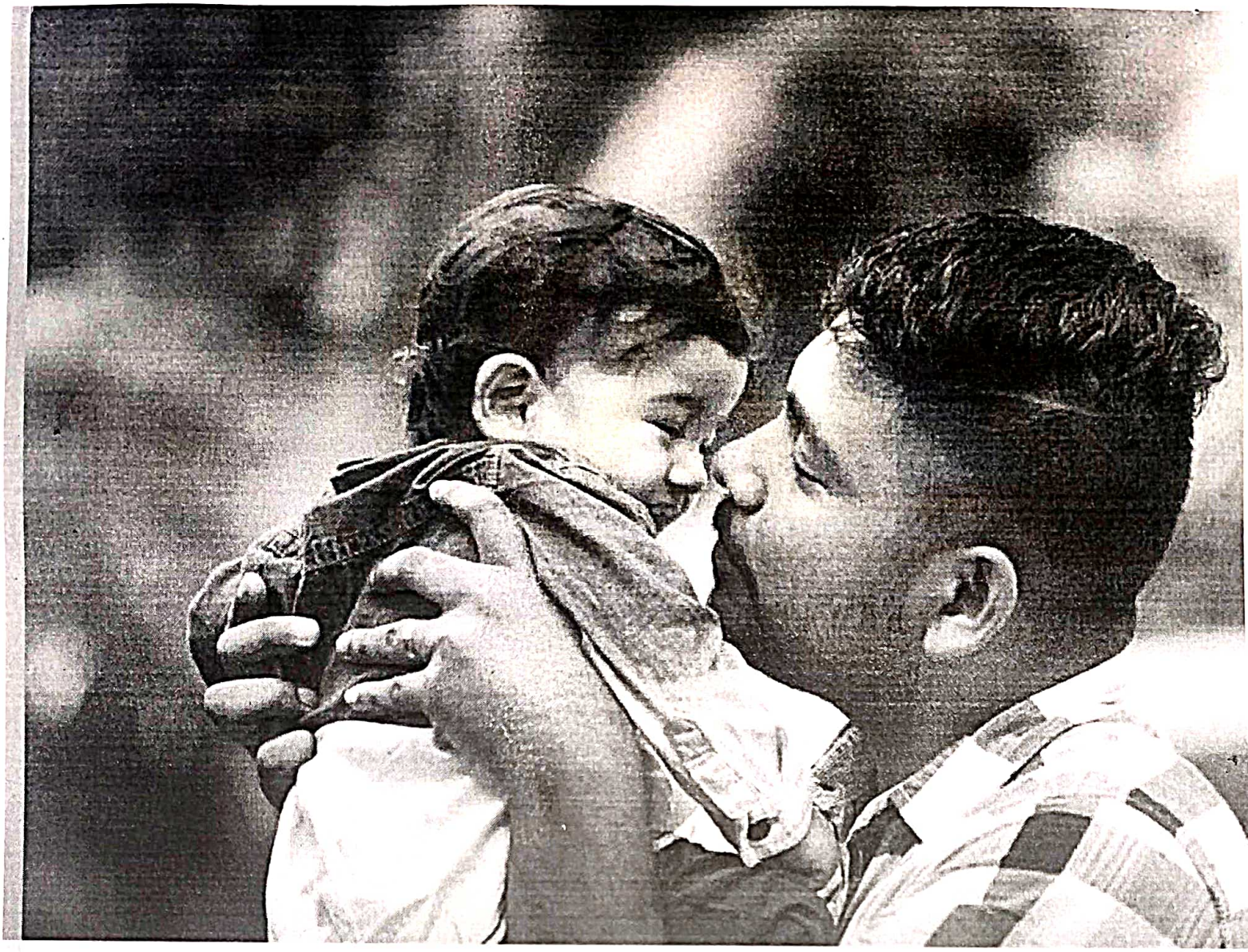
ACUTE INFECTION ACCOUNTS FOR

5–20%

OF ALL CASES OF HIV INFECTION AMONG PERSONS SEEKING TESTIN

This acute phase of infection is associated with peak levels of viremia and a high risk of transmission.

By quickly and accurately identifying HIV-positive patients earlier, this will increase case finding and facilitate fast and appropriate linkage to care.



FOR MORE INFORMATION, CONTACT YOUR LOCAL REPRESENTATIVE OR VISIT ABBOTT.COM/POCT.

1. McCutchan FE; Henry M. Jackson Foundation (Rockville, Maryland). IAVI Report. http://www.pbs.org/wgbh/pages/frontline/aids/atlas/clade.html. Published August 2003.
2. Abbott. Data on File. Determine™ HIV Early Detect Product Insert.
3. Branson B, Stekler JD. Detection of Acute HIV Infection; We can't close the window. *Journal of Infectious Disease*. 2012;205:521-4.

Abbott