

individual Pima CD4 test cartridge using a built-in reagent control. If reagent validation results fall outside the limits set during manufacturing an error message is displayed by the Analyser.

- **Instrument function:** Fluorescent control features on the Pima CD4 test cartridge are analysed to determine functionality of the Pima Analyser. If fluorescent control results fall outside the limits set during manufacturing an error message is displayed by the Analyser.

A number of additional checks are performed during the analysis. If any check is not passed the Pima Analyser will display an error message. Please refer to the Pima Analyser User Guide for error message details.

Limitations

1. The results of a Pima CD4 test should be evaluated in the context of all the clinical and laboratory data available. In those instances where the laboratory results do not agree with the clinical evaluation, additional tests should be performed accordingly.
2. The Pima CD4 test has been evaluated with capillary whole blood and venous whole blood using EDTA as anti-coagulant. Serum, plasma and whole blood obtained using other anti-coagulants have not been evaluated and should not be used.
3. Absolute T-helper cell counts may differ between laboratories using different manufacturer's equipment.

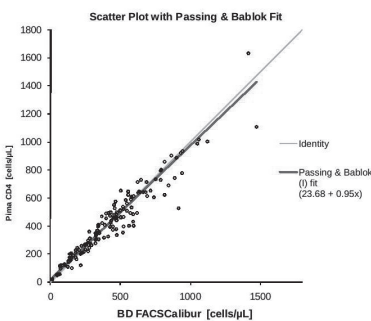
Performance Characteristics

Performance characteristics of the Pima CD4 test were established by testing at Clondiag GmbH in Jena, Germany and at external clinical

sites in Africa and Germany.

Accuracy

The accuracy of the Pima CD4 test for absolute T-helper cell counts was assessed by comparison to the BD FACSCalibur system as reference method. Venous whole blood samples from 149 HIV-positive adult individuals presenting to health care facilities in Uganda were collected and analysed in duplicate using Pima CD4 and the reference method (single measurement). Passing-Bablok regression analysis was performed on the first Pima CD4 cell count measurement versus the BD FACSCalibur cell count measurement and the results are illustrated in the plot below. Total range of all analysed samples was 12 to 1472 cells/ μ l for BD FACSCalibur and 17 to 1631 for Pima CD4. The slope (95% CI) was 0.95 (0.91 to 0.99) with an intercept (95% CI) of 24 (8.5 to 37). The Pearson correlation coefficient (95% CI) between the two measurements was 0.96 (0.94 to 0.97).



Bland-Altman difference analysis (Pima CD4 – BD FACSCalibur) was also performed on the data. The mean bias (95% CI) across all 149 samples was -10 (-22 to 3) cells/ μ l.

Clinical Agreement

Clinical agreement between the methods was assessed about a diagnostic cutoff by computing a two-by-two contingency table. The results are illustrated in the tables below for two diagnostic cutoffs, 200 cells/ μ l and 350 cells/ μ l, respectively. Discordance between the methods at each diagnostic cutoff was assessed for bias using a McNemar test and the bias was shown to be insignificant.

Clinical Agreement about Cutoff > 200			
	Value	95 % LCI	95 % UCI
Agreement	0.953	0.906	0.981
	Pima Negative	Pima Positive	
BD Negative	25	5	
BD Positive	2	117	
Two-Sided McNemar Test (2 out of 7):			
p-value	0.453		
Clinical Agreement about Cutoff > 350			
	Value	95 % LCI	95 % UCI
Agreement	0.940	0.888	0.972
	Pima Negative	Pima Positive	
BD Negative	57	6	
BD Positive	3	83	
Two-Sided McNemar Test (3 out of 9):			
p-value	0.508		

Precision Analysis on Clinical Samples

The within method standard deviation and % CV of the Pima CD4 measurements was calculated based on the duplicate measurements made on all 149 samples and for subsets of samples in the cell count ranges of 0–200, 0–350 and >350 as illustrated in the following table. MEAN is the mean of all duplicate measurements within each range, SD is the root-mean-square of the standard deviations of the duplicate measurements within each range (together with its 95% CI).

Range (cells/ μ l)	N	MEAN (cells/ μ l)	SD (cells/ μ l)	95 % LCI	95 % UCI	% CV
0–200	29	119	20	16	27	16.6
0–350	59	198	23	19	28	11.6
>350	90	580	41	35	47	7.0

Capillary Whole Blood

Performance of Pima CD4 with capillary whole blood samples was shown to be comparable to that with venous blood. Samples from 49 HIV-positive adult individuals presenting to 2 health care facilities in Germany were collected. Venous blood from the same individuals was used for reference testing with BD FACSCalibur. Total range of all analysed samples was 160 to 1181 cells/ μ l for BD FACSCalibur and 167 to 1011 for Pima CD4. Compared to BD FACSCalibur, regression analysis showed a slope (95% CI) of 0.85 (0.76 to 0.94) with an intercept (95% CI) of 46.42 (-5.92 to 98.76). The Pearson correlation coefficient (95% CI) between the two measurements was 0.94 (0.89 to 0.97).

Lot-to-Lot Reproducibility

10 Aliquots of 2 whole blood samples above and below the 350 cells/ μ l cutoff were assessed using 3 different Pima CD4 cartridge lots. The lot-to-lot reproducibility is shown in the table below:

Sample	Mean (cells/ μ l)	Mean % CV
1	267	9.54
2	505	7.05

Cross Reactivity

The CD4 antibody reacts with monocytes as well as with helper/inducer T lymphocytes^{5,6}. The CD3 antibody reacts with all mature T lymphocytes^{7,8}.

Linearity

Linearity was assessed by testing serial dilutions of 5 specimens covering the clinically relevant range of T-helper cells in whole blood. Pima CD4 was demonstrated to be linear from 3 to 2168 cells/ μ l. All data fit the linear regression line with a minimal R² of 0.99. The data is summarized in the following table:

Sample	Range (cells/ μ l)	Slope	R ²
1	3–2168	1.00	1.000
2	4–1507	0.99	0.995
3	4–690	1.02	0.996
4	4–402	1.01	0.997
5	9–391	0.98	0.997

References

1. Pattanapanyasat K and Thakar MR, CD4+ T cell count as a tool to monitor HIV progression & anti-retroviral therapy. Indian J Med Res 2005; 121:539-49.
2. Rachlis AR and Zarowny DP. Guidelines for antiretroviral therapy for HIV infection. Canadian HIV Trials Network Antiretroviral Working Group. CMAJ 1998; 158:496-505.
3. Clinical and Laboratory Standards Institute. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard-6th Edition H03-A6 Vol. 27 No 26.
4. Clinical and Laboratory Standards Institute. Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard-6th Edition H04-A6 Vol. 28 No 25.
5. HCDM (former HLDA VIII) Meeting May (2006), Québec, Canada; WS Code M241.
6. Millan J, Cerny J, Horejsi V, Alonso MA (1999). CD4 segregates into specific detergent-resistant T-cell membrane microdomains. Tissue Antigens. Jan; 53(1):33-40.2.
7. Knapp, W., B. Dorken, et al. Eds. (1989),

- Leucocyte Typing IV: White Cell Differentiation Antigens Oxford University Press. New York.
- 8. McMichael, A.J., P.C.L. Beverly, et al. eds. (1987). Leucocyte Typing III: White Cell Differentiation Antigens. Oxford University Press. New York.

Index of Symbols

-  CE Mark
-  In vitro diagnostic medical device
-  Consult instructions for use
-  Catalogue number
-  LOT number
-  Use by
-  Manufacturer
-  Warning
-  Do not reuse
-  Contains sufficient for <n> tests
-  Temperature limitation 2–30 °C
-  To keep dry

Warning: Pima CD4 cartridges contain small amounts of human blood cell preparations. These cell preparations have been tested and are non-reactive for: Anti-HCV, Anti-HIV1 and 2, HBsAg, Anti-HBc (IgG, IgM), Syphilis, irregular AK, HCV- and HIV-PCR. A residual, but small risk for infection may remain.

© 2009 Inverness Medical. All rights reserved. Pima is a trademark of Inverness Medical group of companies.

For Technical Support please contact your local distributor or call the respective number for your region: Europe: +49.3641.3111-444, Africa: +27.11.450 4411

CLONDIAG GmbH, Lößstedter Straße 103–105, D-07749 Jena, Germany, www.pimatest.com

pima™

CD4
English

Intended Use

Pima CD4 is an automated, image-based immune hematology test intended for the rapid in vitro quantitative measurement of CD3+/CD4+ T cells (T-helper cells) in capillary or venous whole blood. Pima CD4 determines the absolute count of CD4+/CD3+ cells and is intended to be used for the ongoing monitoring of absolute CD4 lymphocyte counts in patients with documented diagnosis of an immunodeficiency disease. The Pima CD4 test is intended for in vitro diagnostic use.

Introduction

Concentration of T-helper cells is an indicator of a patient's immune-status and absolute T-helper cell count decreases in immunodeficient patients. The enumeration of absolute numbers of T-helper cells is an essential part of initial staging of immune status, monitoring the course of immunodeficiency over time, and the response to treatment^{1,2}.

Test Principle

The Pima CD4 test comprises a disposable Pima CD4 test cartridge and the Pima Analyser, and enables the determination of absolute counts of T-helper cells in whole blood. The disposable Pima CD4 test cartridge is equipped with means to take up approximately 25 µl of sample and contains dried reagents needed to perform the test. The Pima CD4 test is performed within the Pima CD4 test cartridge and no part of the Pima Analyser has contact with the sample at anytime in the testing process. This minimises the risk of Analyser contamination and sample carry-over between measurements. After insertion of the Pima CD4 test cartridge into the Analyser, peristaltic movement first transports the sample into the incubation compartment where the sample

interacts with specific antibodies labelled with two different fluorescent dyes emitting light at two different wavelengths (dye 1 and dye 2). One antibody is an anti-human CD3 monoclonal antibody conjugated to dye 1. The second antibody is an anti-human CD4 monoclonal antibody conjugated to dye 2. After a defined incubation time, the stained sample is transferred into the detection channel of the cartridge. The Pima Analyser is equipped with miniaturised multi-colour fluorescence imaging optics. Fluorescence signals are detected by an on-board camera and analysed using proprietary software algorithms on board an embedded computer. T-helper cells carry both CD3 and CD4 surface antigens and therefore emit light at wavelengths specific for both antibody-dye conjugates. This allows the specific differentiation of T-helper cells from other blood cell types carrying only one of the two surface antigens. Results are displayed by the Pima Analyser as cells/µl. Results are also stored in an on-board archive and are assigned to a sample ID that has been entered into the Pima Analyser by the operator and the date/time the test was carried out. Data can be retrieved and down-loaded by the operator at any time after the test. An external Pima Printer can be attached via USB to the Pima Analyser to print test results.

Package Contents and Storage

Materials Provided

Each Pima CD4 box contains:

- 100 individually pouched test cartridges
- 1 package insert

Storage

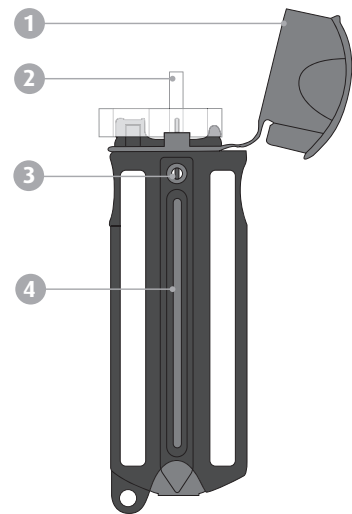
Store at 2–30 °C.

Materials Required But Not Provided

- Pima Analyser
- volumetric or transfer pipette (for venous blood samples only)
- sterile lancets* (for capillary blood samples)
*recommended: Safety-Lancet Super, Sarstedt, Germany. For order information, please view at www.pimatest.com. If other lancets are used, please refer to the specific instructions from the legal manufacturer.
- alcohol swabs
- dry swabs

Pima CD4 Test Cartridge

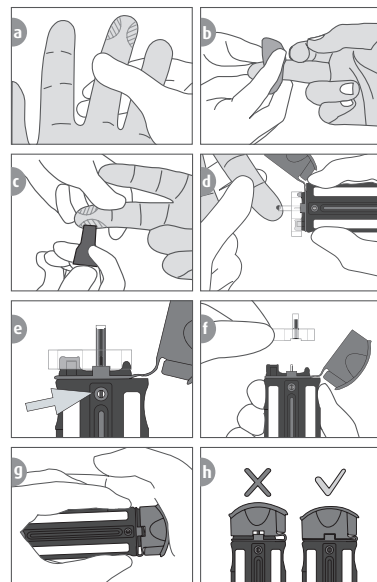
The Pima CD4 test cartridge consists of a solid cartridge base of black plastic, an orange plastic cap ①, a Sample Collector to apply blood sample ②, a control window to check sufficient sample volume ③, a detection channel with a transparent detection channel lid ④, silicone tubing on the back to enable liquid movement in the cartridge and an orange back cover (both not shown in the image).



Precautions

- ! Allow cartridge to reach ambient temperature before opening the foil pouch.
- ! **DO NOT** open the foil pouch until ready to test.
- ! Remove cartridge from foil pouch carefully without touching the detection channel lid.
- ! **DO NOT** use cartridges that have become wet.
- ! **DO NOT** use cartridges if the foil pouch has been damaged.
- ! Properly dispose of all contaminated waste according to federal, state, and local regulations or incinerate.
- ! **DO NOT** use cartridges beyond the expiration date printed on the pouch label.
- ! **DO NOT** remove the Sample Collector until cartridge is loaded with blood.
- ! **DO NOT** attempt to separate the orange plastic cap from cartridge base.
- ! **DO NOT** close the orange plastic cap until cartridge is loaded with blood.
- ! **DO NOT** touch the transparent detection channel lid. Damaged detection channel lids can lead to an instrument error.
- ! **DO NOT** touch or attempt to stretch the silicone tubing. Damaged tubing can lead to an instrument error.
- ! **DO NOT** attempt to remove orange back cover of the cartridge.
- ! **DO NOT** open desiccant bag included in the foil pouch.

Note: White residue visible within the EDTA capillary **DOES NOT** indicate a defective cartridge. This is a result of the normal manufacturing process. The residue will dissolve when in contact with the blood sample.



Sample Collection

Whole Blood Collection By Fingerstick⁴ (please refer to the workflow illustration)

1. Prepare patient for fingerstick sample collection (see illustration a).

The best locations for fingersticks are the 3rd and 4th fingers of the non-dominant hand. Do not use the tip of the finger or the centre of the finger pad. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash. Avoid fingers with rings on.

2. Warm up the fingers if needed. Have the patient hold their hand downwards to increase blood flow to the finger.
3. Wipe the tip of the appropriately selected finger with an alcohol swab and let the alcohol air dry (see illustration b).
4. Remove one Pima CD4 test cartridge from its foil pouch and open the orange plastic cap to fully expose the Sample Collector. **Retain the foil in case Analyser cannot read the cartridge barcode.**
5. Use a sterile lancet to make a skin puncture just off the centre of the finger pad (see illustration c). The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges. It is important to press lancet firmly onto the finger and maintain contact while ejecting the lancet. Do not squeeze or apply strong repetitive pressure (milking) to the site; this may result in hemolysis or tissue-fluid contamination of the specimen. If necessary, gentle massaging of the finger may be conducted in order to ensure a steady blood flow.
6. Wipe off the first drops of blood with a dry cloth or gauze. Ensure steady blood flow that generates large enough drops of blood. If necessary, wipe off another drop, until blood flows freely.
7. Allow blood to flow freely from the pricked finger directly into the Sample Collector (see illustration d) by holding the cartridge at a 45 degree angle for sample loading. Wait until the collector capillary is completely filled with blood. Then remove cartridge from the finger and apply direct pressure to the wound side with a clean dry swab.
8. Hold the cartridge upright and observe the control window to ensure sufficient sample loading (see illustration e). Enough blood is

- applied when the capillary visible in the control window is filled with blood.
9. Squeeze the clip of the Sample Collector between thumb and index finger and remove Sample Collector from the cartridge in one continuous upwards motion (see illustration f). Dispose as biohazardous waste.
10. Completely close orange plastic cap (see illustrations g and h).
11. It is recommended to insert a Pima CD4 test cartridge into the Pima Analyser within 1 minute (but no longer than 5 minutes) after loading a blood sample.

Whole Blood Collection By Venipuncture

1. Collect blood aseptically by venipuncture into a sterile EDTA (ethylenediaminetetraacetic acid) blood collection tube.
2. Invert collection tube 8–10 times.
3. Store at ambient temperature (18–28 °C). The sample must be analysed within 36 hours of draw.
4. Before withdrawing sample for testing, invert collection tube 10–15 times to ensure sample mixing.
5. Use volumetric or transfer pipette to apply blood sample into the Sample Collector of the Pima CD4 test cartridge.
6. Continue as described from point 8 of the previous section.

Test Procedure

1. Add sample to the Pima CD4 test cartridge (see Sample Collection for details).
2. Select «Run Test» on the Pima Analyser.
3. Insert the Pima CD4 test cartridge into the Pima Analyser in the direction indicated by the arrow on the cartridge. Follow on-screen instructions or refer to the Pima Analyser User Guide for details

on how to proceed with the analysis.

4. Remove the Pima CD4 test cartridge when prompted by the Pima Analyser and read result.

Results

The results are calculated automatically by the Pima Analyser. The absolute T-helper cell count is displayed in the first of four result windows of the Analyser. The operator has the option to print a result via an external Pima Printer. For additional information, please refer to the Pima Analyser User Guide.

Quality Control and Acceptability of Results

Built-in Quality Control Features

The Pima CD4 test cartridge contains built-in control features to check Analyser and reagent functionality. The following checks are performed automatically once the cartridge is inserted into the Pima Analyser:

- **Expiry date:** The linear barcode on the Pima CD4 test cartridge contains expiry date information which is checked by the Pima Analyser prior to analysis. If the cartridge is past its expiry date the analysis will not start and the cartridge is rejected. If the Pima Analyser cannot read the barcode, the numeric code can be entered manually by the Operator. This code is printed on the cartridge pouch below the linear barcode.
- **Sample volume:** The Pima Analyser checks whether sufficient sample has been loaded onto the Pima CD4 test cartridge. If insufficient sample has been loaded onto the test cartridge the analysis will not start and the cartridge is rejected.
- **Reagent validation:** The stability of the test reagents is validated for every