# Accellix CD64 Cartridge Instructions for Use (IFU)

*SPC-015 Accellix CD64 Instructions for Use (IFU): CE Mark Version 3.0*

*Version created 10 May 2016*

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## Intended Use:


The Accellix-CD64 system is intended for use in the measurement of leukocyte neutrophil CD64 expression and intended for use in conjunction with other laboratory findings and clinical assessments to aid in the in vitro diagnosis of infection in ICU patients.

## Introduction:

The Accellix platform is a diagnostic system for *in vitro* diagnostic use. It is composed of a tabletop instrument and a single use disposable Accellix-CD64 Cartridge. The instrument enables complete automation of a 3 step process: biochemical sample preparation (*i.e.* the assay), flow cytometer reading and analytical data processing utilizing a proprietary algorithm and in-process software quality controls. The entire test is processed within 26 minutes with no user setup or adjustment requirement. The subject's blood sample is loaded into the assay cartridge (capillary loading) and the cartridge is inserted into the instrument by the user. Then, the instrument automates the sample preparation within the cartridge.

## Test Principle:

CD64 is a high-affinity FCγRI receptor. It is constitutively expressed on monocytes, but its expression on heathy neutrophils is very low (<1000 molecules/cell). The increase of neutrophil CD64 expression is one of the most rapid markers of bacterial infection inflammation, expressed on the cells within 4-6 h. The same change is observed in response to documented infection or tissue injury. Consequently, the measurement of neutrophil CD64 expression is considered to correlate with the presence of inflammation, infection or tissue injury in humans.

The Accellix-CD64 cartridge uses a mixture of four monoclonal antibodies with specificities to CD64, CD45, CD163, and CD15. The use of two antibodies to different epitopes of CD64 enhances the signal to noise ratio of the assay and provides a mechanism for minimizing lot-to-lot variation in the reagent fluorescence signal. The antibody against CD45 stains all the WBC; anti-CD163 and CD15 are used for identification of, respectively, monocytes and neutrophils. Monocytes express CD64 constitutively, and lymphocytes do not express CD64. Thus, the $CD45^{dim}CD15^{+}$ population is identified as neutrophils, i.e. the test population; the $CD45^{+}CD163^{+}$ is identified as monocytes, which are used as an intrinsic positive control; and $CD45^{+}$ only population (lymphocytes) is used as a negative control. The Dragon Green (DG) beads are distinguished from cell populations as $DG^{+}CD45^{-}$. They are used as an intrinsic standard. The use of automated software eliminates the variation inherent in the standard subjective flow cytometric list-mode analysis.

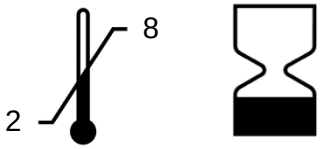
Using a fluorescence bead suspension for standardization of the cellular CD64 quantification provides a mechanism to minimize lot-to-lot differences in the Accellix-CD64 cartridges. The results of the Accellix-CD64 cartridge test are reported as a PMN CD64 Index, which correlates the level of neutrophil activation relative to the control beads.

## Package Contents and Storage:

Single use cartridges with blisters loaded with reagents:

- Blister A contains a mixture of fluorescently labeled mouse monoclonal antibodies against human CD64, CD45, CD163, and CD15 in the proprietary buffer, containing 0.255% bovine serum albumin (BSA) and 0.01% sodium azide.
- Blister C contains control fluorescent beads in the proprietary buffer, containing 0.01% sodium azide and 0.01% Tween 20.

### Storage:

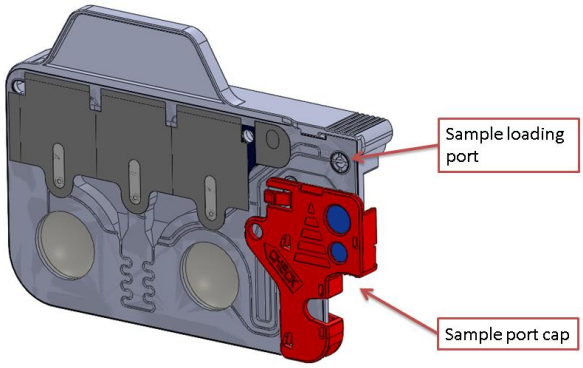



- Store cartridges upright at 2-8°C, when not in use.
- Unopened cartridges are stable until the indicated expiration date.
- Protect the cartridge from freezing, from temperatures above 30°C, and from prolonged time at room temperature (18-26°C). Recommended storage temperature is 2-8°C.

### Shelf Life:

- The cartridge shelf life is 1 year when kept at 4°C. The expiration date is indicated on the cartridge.

## Description of Cartridge:




The single-use Accellix-CD64 cartridge enables the tested sample to be biochemically processed and delivered to the reading cuvette for flow cytometry reading. The cartridge is assay specific as it is able to perform a single assay defined by the reagent compositions, volumes, and activation parameters defined in software. All the reagents are self-contained within the cartridge during the entire assay. The cartridge includes a sample port, a sample port cap, and a backbone containing the reagents and a reading cuvette.

## Special Equipment Required:

Use a blood handling equipment which enables safe and reliable delivery of a sample of 30 to 50µL of EDTA anti-coagulated whole blood (pipette, capillary, blood dropper).

## Specimen:

The Accellix-CD64 cartridge requires from 30 to 50µL (maximum) of EDTA anti-coagulated whole blood. Specimens remain acceptable when stored in an EDTA anti-coagulated vial for up to 6 hours when held at room temperature (18-22°C). Cold storage of blood samples is not recommended.

## Precautions and Warnings:

- Never pipette by mouth and avoid contact with skin and mucous membranes.
- The Accellix-CD64 cartridge should be used only with the Accellix reader instrument.
- Do not use a cartridge if it, or its packaging, is damaged.
- Do not use an expired cartridge. The expiration date is located on the cartridge label.
- Do not attempt to re-use a cartridge.
- Do not puncture the cartridge reagent blisters.
- Handle the cartridge with designated handle or from the outer perimeter wall
- Do not interfere with the assay by opening the instrument door during the test since it will abort the assay and the cartridge will not be re-useable
- Specimens, used cartridges and all materials coming in contact with the specimens should be considered potentially infectious and disposed of with proper biohazard precautions.

## Potentially Hazardous Reagent Components:

The blister reagents contain sodium azide (<0.1% w/ v). This chemical is a dangerous compound when combined with acids or metals. Handle with appropriate care. Solutions containing sodium azide should be disposed of properly.

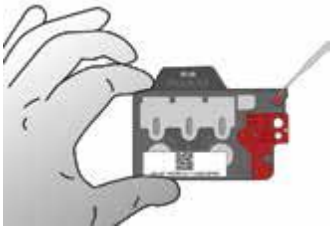
## Test Procedure:

1. Turn the Accellix instrument on, according to the Accellix instrument User Manual. Press the start button displayed on the touchscreen.

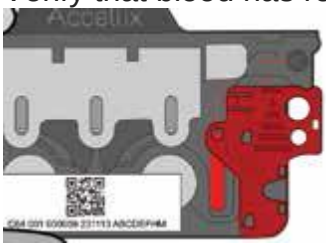
2. Enter the patient ID.

3. Take a new cartridge out of cold storage and wait 10 minutes. If damaged, discard the cartridge and use another one. Lay flat on counter.

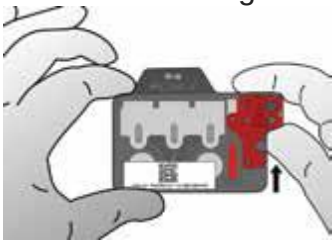
4. Place a drop of blood in the cartridge sample port.



5. Verify that blood has reached checkpoint on cap.



6. Close the cartridge cap, and ensure that it clicks closed. Use cartridge within 2 minutes of adding blood.



7. Insert the cartridge in the instrument.

8. Close the device door.

9. Wait 30 minutes while the instrument performs the assay. The result is displayed on the screen.

10. Remove the cartridge and discard it as a biohazard.

## Results: Principle of the Method & Mathematical Approach

The result of the Accellix-CD64 cartridge test is reported as a PMN CD64 Activation Index, which represents the level of neutrophil CD64 based on fluorescence relative to the fluorescence level of control beads.

The value is calculated as the ratio of the median of the fluorescence intensity of the events detected as PMN to the median of the fluorescence intensity of the events detected as control beads.

$$\text{CD64 PMN Index} = \frac{\text{Median Fluorescence Intensity of events identified as PMN}}{\text{Median Fluorescence Intensity of events identified as control beads}} * \text{Lot Correction factor}$$

The reference range of the PMN CD64 Index for healthy normal blood samples is anticipated to be ≤1.00 with 95% confidence.

Studies have shown that the more severe the systemic acute inflammatory response, the higher the PMN CD64 Index value will be measured.

The anticipated reference ranges for the Monocyte and Lymphocyte CD64 Indices have not been established and are not delivered to the user as results for this test.

## Quality Control & Acceptability of Results:

The assay utilizes autologous cell populations within the patient specimen for both positive and negative controls, which optimizes assessment of pre-analytical variables. The lymphocyte $CD45^+$ only population serves as a negative control population and insures the addition of proper reagents and may detect the presence of fluorescent substances within the diagnostic specimen. The monocyte $CD45^+CD163^+$ population serves as a positive control for assurance of proper specimen staining with reagents from Blister A. Pass/Fail criteria for run are triggered by the software. So internal assay controls (included for instrument calibration and standardization of the cellular CD64 quantification) as well as data controls verify data integrity and maintained analytical performance per each run.

## Limitations:

- Therapeutic use of interferon-gamma, G-CSF, or other agents that modulate the innate immune response, are known to affect the up-regulation of leukocyte CD64 expression and therefore will affect the interpretation of the assay. **Accellix-CD64 cartridge results should not be used as a sole measure of inflammation in these patients, but may be used to monitor the effect of such treatments.**

- Samples from severely leukopenic or neutropenic blood cannot be analyzed by the Accellix software without significant reduction in the accuracy and precision of the PMN CD64 index because of the low number of the cell types to be measured. If the number of events detected is too low the instrument will not provide a result.

## Performance Characteristics:

Normal Range

The normal range was established for the predicate test Leuko64 developed by Trillium Diagnostics (Maine, US)[1, 4]. The CD64 index was calibrated so that a healthy individual would have a 95% probability of having a CD64 index below 1.

LOD (Limit of Detection)

For **limit of detection (LOD)** calculation the mean and the standard deviation (SD) of healthy PMN CD64 indices was calculated. The LOD was calculated as

$LOD = LOB + 1.645 \times SD_{heathy\ PMN\ index}$

According to this method, the **limit of detection** (LOD) of Accellix CD64 index is 0.48.

LOB (Limit of Blank)

For **limit of blank (LOB)** calculation, the mean and the standard deviation (SD) of lymphocyte CD64 indices was calculated. The LOB was calculated as $LOB=Mean_{Lym\ index}+1.645\times SD_{T\ cell\ index}$

According to this method the **limit of blank** (LOB) of Accellix CD64 index is 0.27.

Method Comparison

The Accellix CD64 test has been compared with two different lots of the prototype Leuko64 assay (Trillium Diagnostics). The PMN indices measured by CD64 test were in good correlation with those measured by both lots of Leuko64 test ($R^2$>0.9 for both lots).

Changes in analytical performance

The system is calibrated so that control beads have a fluorescence value on the channel relevant for the assay of around 500 A/D count. The data analysis software includes control criteria that check that Control bead MFI is within target levels. This ensures that the system catches drifts that could happen to laser intensity, detector response or alignment of the system.

Measurement Precision

Repeatability: run-to-run variability has a CV of 10%

Reproducibility: Machine-to-machine variability has a CV of 10%

Diagnostic Performance

A meta-analysis on CD64 [15] summarized the diagnostic specificities in the range of 70-86% (median 76%), and the specificities 85-95% (median 91%).

Measurement Interval

Performance characteristics have been validated for CD64 indices of 0.48-19.2.

## Error Messages:

Error Messages are classified in two groups:

1. **Group 1 errors**: Recoverable errors

   **The system detects that an operation failed and it can be recovered.**

Examples of recoverable errors are:

- → The system does not recognize the cartridge barcode.
- → The cartridge is out of date.
- → Analysis of the sample fails.

Specific instructions for each error type are provided on the instrument screen.

2. **Group 2 errors**: Possibly recoverable errors requiring instrument self-check

   **The system detects that an operation failed. The failure may be related to a hardware failure.**

- → The system provides an error message about which test condition failed and requests that the user perform a self-check.

The self-check program is the same automated procedure which is performed by the instrument automatically upon power-up or by automatic scheduling every 24 hours if the system is not powered down once a day. This self-check procedure checks critical components (such as laser, detectors, cartridge actuation motors and communication between internal components).

→ Upon completion of a successful self-check:

The system returns to the Home screen to allow the user to repeat the test by running another cartridge (with the same or a new sample).

The system may still provide an error message but it could be related to a specific sample that cannot be measured for the test being run. (Please refer to cartridge IFU for the specific assays being run which provides details about Test Limitations and Interferences such as sample types and medical conditions that may lead to assay failure).

→ If the self-check fails:

An error message provides information about which component is failing the self-check test and requests the user to contact service.

Specific instructions for each error type are provided on the instrument screen.

## Disposal:

Specimens, used cartridges and all materials coming in contact with the specimens should be considered potentially infectious and disposed of with proper biohazard precautions, in accordance with local regulations.

## References:

1. *Davis BH. Improved diagnostic approaches to infection/sepsis detection. Expert Rev Mol Diag 2005;5:193-207.*
2. *Schiff D, Rae J, et al. Increased phagocyte CD64 expression and improved Fc-receptor mediated phagocytosis following in vivo recombinant human interferon-γ treatment of normal human subjects. Blood 1997;90:2987-94.*

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4. *Davis BH, Bigelow NC, et al. Neutrophil CD64 expression: potential diagnostic indicator of acute inflammation and therapeutic monitor of interferon- therapy. Lab Hematol 1995;1:3-12.*
5. *Guyre PM, Campbell AS, et al. Monocytes and polymorphonuclear neutrophils of patients with streptococcal pharyngitis express increased numbers of type I IgG Fc receptors. J Clin Invest 1990;86:1892-1.*
6. *Ng PC, Lam HS. Diagnostic markers for neonatal sepsis. Curr Opin Pediatr 2006;18:125-31.*
7. *Davis BH, Bigelow NC. Comparison of neutrophil CD64 expression, manual myeloid immaturity counts, and automated hematology analyzer flags as indicators of infection or sepsis. Lab Hematol 2005;11:137- 47.*
8. *Leino L, Sorvajarvi K, et al. Febrile infection changes the expression of IgG Fc receptors and complement receptors in human neutrophils in vivo. Clin Exp Immunol 1997;107:37-43.*
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10. *Van der Meer W, Pickkers P, et al. Hematological indices, inflammatory markers and neutrophil CD64 expression: comparative trends during experimental human endotoxemia. J Endotoxin Res 2007;13:94-100.*
11. *Groselj M, Ihan A, Derganc M. Neutrophil and monocyte CD64 and CD163 expression in critically ill neonates and children with sepsis: comparison of fluorescence intensity and calculated Indexes. Mediators Inflamm 2008; 2008:202646.*
12. *Bhandari V, Wang C, et al. Hematologic profile of sepsis in neonates: neutrophil CD64 as a diagnostic marker. Pediatrics 2008;121:129-134.*
13. *Hoffmann JJ. Neutrophil CD64 as a sepsis biomarker. Biochemica Medica 2011;21:282-90.*
14. *Icardi M, Erickson Y, Kilborn S, et al. CD64 Index provides simple and predictive testing for detection and monitiroing of sepsis and bacterial infection in hospital patients. J Clin Microbiol 2009;47:3914-19.*
15. *Cid, J., et al. Neutrophil CD64 expression as marker of bacterial infection: a systematic review and meta-analysis. J Infect. 2010;60:313-9.*

## Index of Symbols:

| Symbol | Used for | Symbol | Used for |
|---|---|---|---|
|  | Manufacturer |  | CE Mark |
|  | EU Representative |  | Do not reuse |
|  | Use By |  | Upper and lower temperature limits |
|  | Batch Code |  | Consult instructions for use |
|  | In Vitro Diagnostic Medical Device |  | Biological Risks |

## Customer Support:

| | |
|---|---|
|  | LeukoDx Ltd.<br>3 HaMarpe St., 3rd Floor, AVX Building<br>Har Hotzvim, Jerusalem 9777403, Israel<br>Tel: +972-2-674-4422 Fax: +972-2-674-4455<br>E-mail: office@leukodx.com |

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