1. CLINICAL RELEVANCE

The Complete Blood Count (CBC) with differential is one of the most frequently requested laboratory tests by health professionals due to the important information it reveals about a patient's general health. It typically includes a red blood cell count, a differential white blood cell count and provides determinations of complete blood count and hematocrit. The white blood cell (WBC) count with differential enumerates the different WBC types and reports them both in absolute and percentage values of the total WBC count. White blood cells or leukocytes are nucleated cells present in the blood and are involved in the immune process. Leukocytes can be divided into 2 main groups: phagocytes and immunocytes. Phagocytes include neutrophils, eosinophils, basophils and monocytes. Phagocytes have the ability to attach to, engulf and release enzymes to eliminate foreign substances. Immunocytes include the lymphocytes and are involved in the production of antibodies. An atypical WBC profile may be associated with infection, inflammation, allergic reactions, neoplasia and toxic substance exposure.

The spinith-BC is a quantitative assay for total WBC, 5-part differential counts, and hematocrit determination from venous and capillary blood samples. The percentage of WBC count is frequently used to screen the patient’s immune system but may not be totally representative of his clinical state. Therefore, it is equally important to look at absolute counts in order to understand if an impairment exists and provide adequate treatment.

The reference ranges for total and 5-part differential counts vary with age1. For this reason, the spinith- values are complimentary results and should always be evaluated considering the general medical condition of the patient.

2. ASSAY SPECIFICATIONS

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method Principle</td>
<td>WBC (Leukocyte) counts: hemolysing agent lyzes the red cells and staining agent dyes the white cells. The total number and 5-part differential white cell counts (absolute and percentages) are obtained by image analysis. Hematocrit: Values (i.e. Packed Cell Volume) calculated based on image analysis of a centrifuged whole blood sample where red blood cells are separated from plasma.</td>
</tr>
<tr>
<td>Sample Type</td>
<td>Whole venous blood samples (EDTA) and whole capillary blood samples of human origin</td>
</tr>
</tbody>
</table>

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3. APPLICATION

The spinith-BC test cartridge is used with the spinith instrument (spinith 02.00) as an in vitro diagnostic medical device for the quantitative determination of white blood cells and hematocrit. The system provides values for a total white blood cell count and 5-part differential, including neutrophils, lymphocytes, monocytes, eosinophils and basophils with hematocrit testing from capillary and venous blood samples.

4. PRINCIPLE

The spinith-BC cartridge is used to determine the total and 5-part differential leukocyte counts as well as hematocrit quantification from a small blood sample (15 µL) in a time period of 15 minutes. The microfluidic cartridge allows for automated sample processing; erythrocyte lysing, white blood cell specific staining and sample centrifugation. The determination of the leukocyte profile is based on a series of images acquired through an optical microscopy module that captures the specific morphology and the cellular components of each cell type and feeds them into a custom developed spinith- system software application. The hematocrit values (i.e. Packed Cell Volume) are also calculated based on image analysis of a centrifuged whole blood sample where the red blood cells are separated from the plasma. The height of the separated red blood cell column is compared to the height of the entire sample column to determine the packed cell fraction ratio.

5. SAMPLE TYPE

The spinith-BC cartridge can be used with venous whole-blood (EDTA) and capillary whole-blood of human origin.

6. SAMPLE PROCESSING AND STABILITY

Sample collection should be performed according to the guidelines of the World Health Organization, available in “Guidelines on Drawing Blood: Best Practices in Phlebotomy” (WHO/EHT/10.01)3. Capillary blood samples should be loaded into the test cartridge immediately after sample collection. After loading a blood sample into a spinith-BC cartridge it should be tested immediately. If the assay is not initiated immediately after
sample loading, clotting factors may alter the test result and the cartridge should be discarded and never tested.

7. **spinit®- BC TEST CARTRIDGES**

7.1 **PACKAGING**

Each package contains the following items:
- Test cartridges: spinit®-BC
- Instructions for Use

7.2 **REAGENTS**

Each test cartridge contains:
- Saponin
- Methylene Blue
- Anticoagulant reagent (EDTA)

7.3 **NECESSARY MATERIALS REQUIRED – NOT SUPPLIED**

- 15 µL capillary tubes. biosurfit recommends using Microsafe® tubes from Safe-tec (catalogue number MS-50)
- Lancets for obtaining capillary samples

7.4 **REAGENT PREPARATION**

The spinit®-BC cartridge is ready-to-use and does not require any reagent preparation step.

7.5 **STORAGE AND STABILITY**

The spinit®-BC cartridge shelf-life is printed on the test package label and on each single cartridge pouch label. The expiry date is valid if the cartridges are stored in closed pouches at room temperature. Once the pouch has been opened the cartridge must be used within 15 minutes. DO NOT FREEZE

7.6 **ACCEPTABLE PERFORMANCE**

The acceptability of the spinit®-BC cartridge result is determined by successful completion of a series of internal checks. The spinit® instrument has a built-in safety mechanism capable of detecting calibration and other errors that may occur during each test protocol in order to minimise the risk of non-detected errors by users.

Note on system reliability

The spinit® system automatically performs a comprehensive set of performance verification routines every time a result is provided. To guarantee the functional performance of the device, anytime the instrument or cartridge do not meet the quality control acceptance criteria, a screen error code message is displayed to help the end user resolve the issue.

8. **CALIBRATION**

8.1 **CALIBRATOR INFORMATION**

Prior to using a spinit®-BC cartridge it may be necessary to update the system memory with specific parameters for each lot produced. Lots have unique identification numbers and are generally coded under bar codes. A bar code reader is provided to this effect.

Under normal operating conditions the system memory may be updated for each lot of cartridges and after maintenance procedures.

9. **QUALITY CONTROLS**

If the user chooses to perform additional Quality Control checks to comply with internal regulations, biosurfit will recommend previously tested and commercially available quality control reagents with the acceptable reference range for the spinit® instrument.

10. **FUNCTIONAL PERFORMANCE**

10.1 **METHOD COMPARISON**

Results from the method comparison study performed according to CLSI Document EP9-A2 on the spinit®-BC system and the Siemens ADVIA 2120 (standard laboratory reference method) for the total and 5-part differential WBC counts and hematocrit values. Regression analysis was used to calculate correlation coefficients (r) of duplicate measurements obtained with clinical whole venous blood samples.

An average of 100 clinical samples were analyzed using different spinit® instruments and spinit®-BC cartridge lots. The majority of tests were performed within the reference ranges cited for normal adults (refer to assay specifications) with some WBC subclasses exhibiting data points in the upper and lower limits of the reference range.

Figure 1 Equivalency of the spinit®-BC test cartridge with the reference method: Siemens ADVIA 2120 for hematocrit quantification
For accuracy and precision determinations, the results were calculated based on the method comparison data. A range of clinical whole blood samples were tested in duplicate on different days with different spin®-BC test cartridges and spin® instruments. A summary of the results is presented in table 1.

<table>
<thead>
<tr>
<th>Test</th>
<th>N</th>
<th>SD</th>
<th>CV (%)</th>
<th>Bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>105</td>
<td>4.8</td>
<td>4.6</td>
<td>-2.2</td>
</tr>
<tr>
<td>WBC Classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>116</td>
<td>5.9</td>
<td>5.1</td>
<td>-2.9</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>100</td>
<td>7.4</td>
<td>7.4</td>
<td>-1.1</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>91</td>
<td>8.0</td>
<td>8.8</td>
<td>-1.4</td>
</tr>
<tr>
<td>Monocytes</td>
<td>100</td>
<td>13.5</td>
<td>13.5</td>
<td>-3.4</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>94</td>
<td>0.06</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

10.2 PRECISION / ACCURACY
11. DIAGRAM – spinit®-BC test cartridge

![Diagram of spinit®-BC test cartridge]

<table>
<thead>
<tr>
<th>Structure</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Detection zone</td>
</tr>
<tr>
<td>2</td>
<td>Sample inlet area</td>
</tr>
<tr>
<td>3</td>
<td>Haematocrit chamber</td>
</tr>
</tbody>
</table>

Design and development, production, installation and servicing of the spinit® products are performed in compliance with Biosurfit’s quality management system, certified by TÜV Rheinland for ISO9001:2008 and ISO13485:2012.

12. REFERENCES

1. Linton, P.J.; Dorshkind, K.; Age-related changes in lymphocyte development and function. Nature Immunology. Volume 5 Number 2 February 2004
11. Practical Haematology; Dacie and Lewis; Elsevier (2006)