Alere INRatio®2 METHOD AND SAMPLE COLLECTION

1. PURPOSE AND SCOPE

The purpose of this document is to describe the procedure for performing an INR test using the Alere INRatio2 analyser. The INRatio2 meter can be used by healthcare professional for measuring INR, as well as by patients for self-testing. This monitoring system is not intended for screening purposes. This method is to be used for the quantitative determination of prothrombin time in fresh capillary and venous whole blood. This test is used for monitoring oral anticoagulant therapy – long-term therapy with Coumarin derivatives.

2. HAZARDS

Patient Samples
All patient samples should be treated as potentially infectious and handled appropriately. Standard precautions should be employed. Personal Protective Equipment should be worn when processing samples, quality control testing and maintenance procedures.

3. CLINICAL SIGNIFICANCE

Oral anticoagulation therapy is the established treatment for patients suffering from a range of conditions in which it is necessary to inhibit the formation of blood clots within the circulation.

The major indications for anticoagulation include:
• Prevention of thrombosis in patients with prosthetic heart valves/stents
• Treatment and secondary prevention of venous thromboembolism
• Primary prevention of venous thromboembolism in high risk patients
• Primary prevention of stroke in patients with atrial fibrillation.

Oral anticoagulant drugs work by antagonizing the effects of Vitamin K and this reduces the blood’s ability to form a clot. This effect can be measured by determining the prothrombin time (PT) in a patient’s blood and comparing it with a standard figure. The resulting ratio is called the Internal Normalised Ratio (INR).

INR is a good indicator of effectiveness and risk of bleeding during Warfarin therapy. Regular INR testing is required to adjust the Warfarin dose in patients to maintain their INR as near to the appropriate target INR as possible. Optimum target INR figures have been established for different diagnoses.

Warfarin is a potentially hazardous drug causing major bleeding in 1-2% of people treated, and intercranial bleeding in about 0.1-0.5% during each year of therapy (Gallus A et al MJA 2000; 172: 600-605). Patients on therapy should be monitored closely.
4. **TEST PRINCIPLE**

A drop of blood is applied to the test strip, where it is drawn into the test area. The blood mixes with reagents that start the clotting reaction. As the blood clots, there is a change in the impedance in the sample. The monitor detects the change and then calculates the PT for the sample and reports the result on the screen.

The reagents used in performing PT tests can vary substantially between testing methods. This may cause differences in test results depending on the method used. For this reason, the International Normalised Ratio (INR) unit was developed. The INR is a mathematical correction of the PT result that adjusts for sensitivity differences in reagents.

The INRatio2 PT Monitoring system provides both a PT and an INR result with every test.

4.1 **Interference**

No interference was observed up to the following concentrations for all analytes:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>513 umol/L</td>
</tr>
<tr>
<td>Triglycerides (lipemia)</td>
<td>16.95 mmol/L</td>
</tr>
<tr>
<td>Fondaparinux</td>
<td>5 mg/L</td>
</tr>
<tr>
<td>Haemoglobin (hemolysis)</td>
<td>10,000 g/L</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>600 µg/L</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>4 mmol/L</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Unfractionated Heparin</td>
<td>2 Units/mL plasma</td>
</tr>
<tr>
<td>Low molecular weight heparin (LMWH)</td>
<td>3 Units/mL plasma</td>
</tr>
</tbody>
</table>

Anti-phospholipid antibodies (APA) such anticardiolipin antibodies or lupus antibodies may falsely prolong coagulation times using the INRatio2 system. Where APA are known to be present, it is imperative that a result be obtained from a laboratory using an APA insensitive method.

**Factor Sensitivity:**

The INRatio/INRatio2 System is sensitive to Factors II, V, VII, and X at the following levels (% of normal factor level):

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor II</td>
<td>&lt;56%</td>
</tr>
<tr>
<td>Factor V</td>
<td>&lt;62%</td>
</tr>
<tr>
<td>Factor VII</td>
<td>&lt;78%</td>
</tr>
<tr>
<td>Factor X</td>
<td>&lt;74%</td>
</tr>
</tbody>
</table>

4.2 **Limitations of the test**

- Only fresh capillary or venous whole blood is suitable.
- Instrument must remain in a horizontal position whilst performing a test.
- Blood collection in glass tubes or syringes or in tubes containing an anticoagulant or separating gel is not suitable and must not be used (vacutainers cannot be used).
- Samples should not be taken from an arm receiving an intravenous infusion.
• Poor blood flow due to poor capillary or venepuncture technique may cause erroneous results.
• Apply the first drop of blood to the INRatio2 test strip. Never add more blood to the test strip after the test has begun or perform another test with the blood from the same puncture site.
• Accuracy validated only for hematocrit values between 25% and 53%.

4.3 Accuracy

This product fulfills the requirements for Directive 98/79/EC on in vitro diagnostic medical devices.

The INRatio2 PT Monitoring System capillary (finger stick) and venous (non-anticoagulated) whole blood results were compared to plasma results obtained on the Sysmex CA-560 system with Innovin thromboplastin (plasma obtained from the same patients). A method correlation between the INRatio2 System and the Dade Innovin on the Sysmex CA 560 System was performed on 1 lot of test strips at 4 external clinical sites. The correlations in these method comparisons were ≥ 0.857.

4.4 Precision

Precision was assessed on capillary (finger stick) and venous whole blood based by duplicate testing on samples from normal and therapeutic subjects within specified INR ranges; testing was by both trained patients (PST) and healthcare professionals in two separate clinical trials.

<table>
<thead>
<tr>
<th>User Type</th>
<th>Subject Type</th>
<th>Normal INR</th>
<th>Therapeutic INR</th>
<th>n</th>
<th>Mean INR</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Professional Capillary Blood</td>
<td>Normal INR ≤ 2.0</td>
<td>18</td>
<td>1.10</td>
<td>0.09</td>
<td>8.18%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional Capillary Blood</td>
<td>Therapeutic INR 2.0 to 4.5</td>
<td>155</td>
<td>2.85</td>
<td>0.17</td>
<td>5.84%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional Venous Blood</td>
<td>Normal INR ≤ 2.0</td>
<td>19</td>
<td>1.07</td>
<td>0.06</td>
<td>7.74%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional Venous Blood</td>
<td>Therapeutic INR 2.0 to 4.5</td>
<td>160</td>
<td>2.88</td>
<td>0.16</td>
<td>5.67%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient Self Tester Capillary Blood</td>
<td>Therapeutic INR 2.0 to 4.5</td>
<td>46</td>
<td>2.78</td>
<td>0.18</td>
<td>6.32%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = number of subjects with duplicate measures.

5. INSTRUMENT
**Product specifications**

### 5.1 Operating Conditions and Technical Data

<table>
<thead>
<tr>
<th>Specification</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range</td>
<td>10°C - 35°C</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>15% - 95% without condensation</td>
</tr>
<tr>
<td>Position</td>
<td>Place meter on a level, vibration-free surface. Do not hold the monitor in your hand while running a test</td>
</tr>
<tr>
<td>Measuring range</td>
<td>0.7 – 7.5 INR</td>
</tr>
<tr>
<td>Sample size</td>
<td>15 µL</td>
</tr>
<tr>
<td>Test time</td>
<td>Approx. 60 seconds</td>
</tr>
<tr>
<td>Memory</td>
<td>120 tests with date and time (60 available for on-screen review)</td>
</tr>
<tr>
<td>Barcode scanner</td>
<td>No</td>
</tr>
<tr>
<td>Interface</td>
<td>RS232 data port. PC Connect - web based allows result download from memory to PC</td>
</tr>
<tr>
<td>Battery operation</td>
<td>4 x AA alkaline batteries</td>
</tr>
<tr>
<td>Mains connection</td>
<td>AC power. Input: 100-240 VAC Output: 7.5 VDC</td>
</tr>
<tr>
<td>Number of tests per set of batteries</td>
<td>Approx. 200 tests</td>
</tr>
<tr>
<td>Safety class</td>
<td>Class II</td>
</tr>
<tr>
<td>Automatic power-off</td>
<td>Yes – After 10 minutes of non-use</td>
</tr>
<tr>
<td>Dimensions</td>
<td>151 x 74 x 46 mm</td>
</tr>
<tr>
<td>Weight</td>
<td>263g (with batteries)</td>
</tr>
</tbody>
</table>

### 5.2 Storage and transport conditions

<table>
<thead>
<tr>
<th>Specification</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range (Meter)</td>
<td>-20°C to +70°C</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>15% - 95% (non-condensing)</td>
</tr>
</tbody>
</table>

### 6. SPECIMEN REQUIREMENTS

#### 6.1 Sample Material

Fresh Capillary or venous whole blood with NO anticoagulants (heparin, EDTA, citrate, oxalate or other substances) added.

**Wash hand with warm soapy water and dry thoroughly prior to collecting samples**
Capillary blood (from finger prick)
- Before performing capillary puncture, stimulate blood flow in the fingertip by warming the hands or soaking the fingertip in warm water. Dry fingertips before proceeding.
- Clean the puncture site.
- Puncture a non-callused area on the side of the fingertip.
- Use a lancing device that provides a deep puncture so that blood flows freely.
- Immediately after lancing, massage gently along the side of your finger to obtain a large drop (at least 15 µL).
  **NOTE:** Squeezing the fingerstick site excessively (milking) may release interstitial fluid into the blood sample. This may cause inaccurate results.
- Immediately and within 15 seconds apply a large, well-rounded drop without air bubbles to the test strip.

Microsafe capillary tube
- A Micro capillary tube is a one-piece collection and dispensing device that can be used for finger stick sampling and dispensing if required.
- Use of the micro capillary tube is optional.

Venepuncture
- Blood must be collected in a plain plastic syringe without using anticoagulants using a 23-gauge needle (approx 0.65 mm) or larger.

7. CARTRIDGES / REAGENTS

7.1 Storage and handling
- Strips should be stored at room temperature (10° - 32°C) until the expiration date indicated on package.
- Strips may be refrigerated at 2° - 10°C until the expiration date indicated on package.
- Allow the foil pouch to come to room temperature for 5 minutes before opening pouch. Do not freeze.
- After removing a test strip from the foil pouch, the test strip must be used within 10 minutes.
- Average shelf life of strips 9 -12 months.

7.2 Storing information about test strips
Every lot of test strips has a code printed on the box and test strip pouch. Each code strip belongs to a single lot number and provides important information about the lot-specific properties of the test strip. On opening a new box of test strips, ensure the code stored matches the code of strips in use.

8. CALIBRATION
Each lot of INRatio2 Test Strips is calibrated to a reference lot of human recombinant thromboplastin traceable to the WHO International Reference
Preparations. The ISI (International Sensitivity Index) for each lot of test strips has been established as 1. Operator calibration is not necessary.

9. QUALITY CONTROL

9.1 Quality control material (perform as per your organisation protocol)
QC is important to ensure the user’s technique, integrity of the strip and performance of the whole system. The INRatio2 meter contains an on board quality control, which is performed automatically with every test.

Each test strip includes two control channels that test a low and a high PT control each time a sample is tested. If the QC result display option is selected, the actual control times will be displayed. Whether or not QC is displayed, INRatio2 system only displays the actual PT/INR results if both QC results are within range for that strip code. If the QC results are out of range, a warning icon and QC LO or QC HI will appear on the display, and no PT/INR results will be reported.

If the monitor still gives that warning the following actions should be checked:
• Test strips are not expired.
• Correct test strip code has been entered.
• Confirm that strips have been stored properly.
• Repeat test if you believe the strips are okay. If you continue to get the QC error message, then make a note of the errors and call Technical Support for assistance.

Enrolling in an External Quality Assurance Program is encouraged to objectively compare results with other users using the same method of testing. If an External Quality Assurance Program is not available, monthly lab comparison is encouraged.

9.2 Laboratory Comparison
If your policy states you must perform laboratory comparison then perform a venepuncture sample for the laboratory and store in a Sodium Citrate (blue top) blood tube. Perform a finger prick and run a sample on the INRatio2. Collection of both the venepuncture and capillary sample should occur at the same time. Record and compare the results ensuring they are in acceptable range for your organisation.

10. TEST PROCEDURE

NOTE: Wash hands with warm soapy water or clean finger with an alcohol wipe prior to testing and allow to dry.

10.1 Performing the test
Preparing the meter
• Remove test strip from refrigerator and bring to room temperature (5 minutes).
• Turn on monitor by pressing OK button.
- The monitor will prepare for testing for a few seconds
- The meter will prompt you to insert a test strip

**Inserting the test strip**
- Insert the test strip so that the clear end with the electrodes goes in first, and make sure the sample well in the strip lines up with the light source on the test strip guide.
- Check that the test strip code displayed on the meter matches the strip code on the test strip pouch. If the code matches, press OK.
- If necessary, change the test strip code by pressing the UP/DOWN buttons. Press OK to confirm digit change and move onto the next. Press OK again to proceed with test.

  **NOTE:** The test strips must be used within 10 minutes of opening the sealed packet.

**Applying the sample**
- The meter will warm up for a test and display the temperature symbol
- The monitor prompts you to apply a drop of blood when the meter beeps and a green sample target light will appear through the test strip sample well.
- Perform a finger prick. Immediately and within 15 seconds apply a large hanging drop of blood to the test strip over the green light.

  **NOTE:** Do not smear the blood onto the test strip. Do not add more blood. Do not touch or remove the test strip during testing.
Results
• The monitor will beep and countdown as it performs a test. 
  **NOTE:** Do not move or touch the monitor while it is running a test.
• The result will appear on the display and will be automatically stored in the meter’s memory. Record result.

10.2 Microcapillary tube
• The device consists of a plastic tube with an air vent that regulates airflow and volume. The plastic tube fills by capillary action to a preset volume, avoiding air bubbles and wasted samples. Pinching the bulbous unfilled portion of the tube can then easily dispense the collected samples. See package insert for directions on dispensing the sample onto the device.

10.3 Venepuncture
• Blood must be collected in a plain plastic syringe without using anticoagulants using a 23-gauge needle or larger.
• Immediately after venepuncture (within 10 seconds) discard the first 4 drops of blood and apply 5th drop to the application well (a large well-rounded drop without air bubbles).

11. RESULTS
The INRatio/INRatio2 System has been configured so that results are reported as International Normalised Ratio (INR). The INRatio/INRatio2 System has a PT reportable measuring range of 0.7 to 7.5 INR units. For results below or above this measuring range the INRatio or INRatio2 Monitor will display “< 0.7 INR” or “>7.5 INR”.

11.1 Non Anticoagulated Range
INR: 0.8 – 1.2

11.2 Therapeutic Range
The anticoagulant effect of Warfarin should be kept at an International Normalised Ratio (INR) of about 2.5 (desirable range, 2.0 – 3.0), although a higher level may be needed in certain clinical conditions. The risk of bleeding increases exponentially with the INR result and becomes clinically unacceptable once the INR exceeds 5.0.
11.3 Unusual Results
An unexpected result may include any result that falls outside the therapeutic range, or a result that falls inside the therapeutic range but is not consistent with the clinical symptoms (e.g., such as bleeding or bruising).

Causes of unexpected results
- Changes in diet, lifestyle or taking nutritional supplements.
- Certain prescription drugs and over the counter medicines (e.g. antibiotics).
- A haematocrit greater than 53% or lower than 25% has not been validated.
- Anti-phospholipid antibodies (APA) such anticardiolipin antibodies or lupus antibodies may falsely prolong coagulation times using the INRatio2 system. Where APA are known to be present, it is imperative that a result be obtained from a laboratory using an APA insensitive method.
- Liver disease, congestive heart failure, thyroid dysfunction, and other diseases or conditions can affect the action of oral anticoagulants and the INR value.

What to do when you get an unexpected result
- If the result is outside the therapeutic range, follow your clinic’s steps for re-testing.
- If, after re-testing, the result is still outside the therapeutic range, consider the above causes.
- An INR ≥ 4 should be confirmed with the laboratory or if not possible then Point of Care INR.
- If the result falls within the therapeutic range, but there is reason to believe the INR could be significantly different (e.g. bleeding or bruising), testing by an alternative method should be arranged immediately.

12. MAINTENANCE

- No maintenance is required other than routine cleaning.
- Clean the outside of the monitor with a clean damp cloth. If necessary, a mild detergent or disinfectant (such as 5% bleach or 70% isopropanol) may be used.
- Clean the area around the test strip guide with a swab or pad that has been dampened with alcohol or 5% bleach solution.
- Ensure the area dry for 15 minutes before testing.
- Do not allow any liquid to spill on the monitor.

13. REFERENCES
This method has been adapted from the Alere INRatio2 Prothrombin Time (PT)/INR Professional Testing System User Guide, and test strip package insert.