

**INSTRUCTIONS FOR USE OF SIMPLE SIMON® PT REAGENT**

Product number ZAF 101 Lot K142M Exp 2012-05

**CE IVD****AREA OF USE**

Simple Simon®PT is designated to point of care analysis of prothrombintime (PT) determination at hospital laboratories, health care centres and doctor's offices. Simple Simon®PT is to be used by personnel trained for the purpose. Signs of appropriate education is knowledge of the concept "coefficient of variation", and to achieve a CV<4% analyzing duplicate samples in the therapeutic range, i.e. INR2-3. The coagulation assay measures the compounded activities of the vitamin-K dependent coagulation factors II, VII and X. This product is intended for laboratory use, "In vitro use only".

**PRODUCT DESCRIPTION**

Simple Simon®PT, is a wet-chemistry based analytical procedure which analyzes PT according to Owren (ref 1). The liquid reagent contains, besides tissue thromboplastin, coagulation factor V and fibrinogen. The PT determination is specific to the vitamin K dependent coagulation factors II, VII and X, and is expressed in international normalized ratio, INR, the expression recommended by WHO. INR is the ratio of measured PT and a normal PT, harmonized to a WHO reference procedure, by an exponent, ISI, international sensitivity index. Innovative methodology, ref 4&5, makes it possible using Simple Simon®PT, to have the same procedure, 10µL sample and 200 µL reagent, to different types of samples. Samples can consist of native blood, citrate anticoagulated blood and plasma. The innovative technology also makes it possible to perform PT analyses at ambient room temperature between 17-45°C. The reader is not thermostated. Simple Simon®PT gives values close to those of hospital laboratories calibrated with approved substances.

When assayed, the sample and reagent is mixed in a relation 1:21 (4,8%). Due to dilution is the method relatively insensitive to heparin.

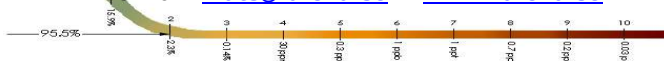
**ANALYTICAL PROCEDURE AND FUNCTION OF READER**

Analytical procedure of Simple Simon is addition, using pipette and disposal tip, of one part sample (10µL) into 20 parts of reagent (200 µL). At sample addition, reagent is placed into a reaction tube, produced of optical high quality plastic, which is placed in the sensor equipped measuring point. Prior to sample addition the light transparency of the reagent is determined. Point of time of sample addition is automatically registered, simultaneously the temperature of reader is recorded. The display shows an instruction to the operator to mix the sample to the reagent by rotating the pipette, with tip attached. Mixing movements are registered automatically and must reach a certain level to allow the assay to proceed. At end of mixing procedure, operator is instructed to remove pipette out of reagent- sample mix. A moment later another measurement of light transmission is recorded. These two light measurements allow an automatic determination of sample type, blood or plasma, and the proportion of blood cells, hematocrit or EVF (erythrocyte volume fraction). Time recording continues with automatic surveillance of changes in light transparency. Recording stops when changes indicate coagulation of the mixture, clotting has occurred. The difference between starting time and registration of coagulation is the clotting time. A period past the clotting time, optical changes are inspected to verify a correct clotting pattern. In the memory of the datachip, lot specific values are stored, these are used together with the determined values of clottingtime, temperature and EVF, to calculate INR- value of the sample (ref 4&5). The INR value of sample, plasma or blood, is shown on the display after a push of button. Native or citrate anticoagulated blood- sample types which not are equivalent due to dilution by anticoagulant- are not automatically separated by the reader. Information on sample type is achieved by pushing the button. The first value to appear on the display is INR of native blood (default), by pushing button a second time values of citrate anticoagulated blood is shown. The reader is calibrated together with the other components of the product – reagent, reaction tube, pipettes and tips- and should be used together.

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## REAGENT PROPERTIES

The reagent of Simple Simon<sup>®</sup>PT is delivered in two portions of components; one dry component which consists of biochemicals in a storage suitable form and one liquid component designed to act as a solvent of the dry component. When one portion of dry component is dissolved by one portion of liquid, a PT reagent is created, which consists of defined levels of thromboplastin from rabbit brain and fibrinogen and FV derived from bovine blood. The PT reagent also has defined levels of free calcium ions, buffering substances and albumin. The reagent will remain stable for one month when stored refrigerated. Guide to optimal reagent handling, see "preparation" below.

## STABILITY AND STORAGE

Pre-calibrated reader, reagent components, reaction tubes, pipette tips and pipettes are delivered as one product. Product and components have the same lot number and expiration date. Expiration date is set by the least stable component, the freeze-dried reagent component. Although none of the components are usable as laboratory PT test when expiry date has passed, and could not be used without compromising the manufacturer's responsibility. A prerequisite is that all product components are stored according to manufacturer's recommendations. Storage recommendations are labelled on each component. The freeze-dried component, together with dilution buffer is to be stored refrigerated (2-8°C), other components are to be stored at room temperature (17-26°C). Storage instructions for reconstituted reagent, see "preparation" below.

## PREPARATION

- 1. Reconstitution of lyophilized reagent.** Fetch one vial of lyophilized reagent and one vial of buffer from refrigeration storage. Pour the cold buffer into the reagent vial. **Ideal reconstitution temperature is between 2 and 8°C.** Immediately, seal the reagent vial with the screw cap from the buffer vial and mix contents by turning vial upside down several times during at least 30 seconds. Check that all content is properly dissolved. Note time and date of reconstitution on the vial label. The reagent should be homogenous and without visible particles, the reagent is a little opaque due to content of microscopic fragments of thromboplastin, and small bubbles coming from the lyophilized biochemicals, the latter will disappear within an hour.
- 2. Dispensing and storage of reconstituted reagent.** Arrange 20 (one vial) or more reaction tubes in a rack, dispense reagent and place stoppers on tubes. The rack, with filled reaction tubes, is to be stored refrigerated (2-8°C). Reagent is dispensed using the green pipette (200µL) with a clean tip attached. If other pipettes are used, check the generated INR values by analyzing the included coagulation control ZAP. Pipette 200µL of reagent into a reaction tube and seal with a clean blue stopper. Place the reaction tubes in a refrigerator within 1-3 hours after reconstitution. It is very important that the reagent not is contaminated, especially not with blood or plasma. The reagent is very sensitive to coagulation factors and contaminating the reagent results in fibrin formation and altering of properties. Recommended storage for reconstituted reagent shall not exceed three weeks. Continuous quality surveillance is possible by using the control ZAP. The recommended coagulation control is ZAP as the control has INR values traceable to international references (RBT/05).
- 3. Reagent handling in connection to analyzing.** To secure that reagent in reaction tube is in correct state and has the same temperature as the reader records, reaction tubes with reagent should be temperature equilibrated by standing in the aluminium plate for **at least 15 minutes** prior to analysis. The time necessary to reach equilibrium increases with decrease of room temperature. To check correct state use the control ZAP.



## WARNINGS AND PRECAUTIONS

**The buffer is harmful. The buffer ZAF 101-2, and subsequently reconstituted reagent contains sodium-azide to prevent bacterial growth and to transform haemoglobin into a form that allows a more precise and accurate determination of EVF. Azide is a quick acting poison and alike cyanid blocks oxygen transport to cells. Do not drink or consume buffer or reagent. Flush with rich amounts of water if buffer or reagent is poured down drains.**

**- Do not drink or consume buffer, ZAF 101-2 or reconstituted reagent ZAF 101-3! The product is health hazardous if consumed.**

**- Do not pour buffer or reconstituted reagent down drains that has pipes of lead or copper, azide reacts with named metals into explosive compounds.**

**Material safety data sheet is provided on demand by the supplier or by Zafena AB.**

**Blood samples may be contagious. Always use protection gloves to prevent infection.**

## THE READER

The reader of Simple Simon<sup>®</sup> PT is a battery driven unit which optically determines coagulation time and EVF. Temperature is measured by a thermistor- bridge. By using stored calibration data the reader calculates the INR value from measured values, see above text "analytical procedure and function of reader", and ref 4&5. The three batteries, size AA, needs to be replaced when battery check function shows "weak battery".

## PRODUCT COMPONENTS, STORAGE AND HANDLING



Simple Simon<sup>®</sup> PT, product ZAF 101, arrives in two packages. One is containing the reader, the other is containing material for consumption, products ZAF 101-2, 101-3.....101-9. Specifications, see text below. The lyophilized reagent, ZAF 101-2, and buffer, ZAF 101-3, arrives in the same package as other articles of consumption, should be separated and stored refrigerated (2-8°C). Product components should not be stored or used in direct sunlight.

Product components: Calibrated reader, ZAF 101-1. Reagent, 10 vials of lyophilized biochemicals, ZAF 101-2. Buffer (solvent) 10 vials of 4mL, ZAF 101-3. White pipette, 10 µL, ZAF 101-4. Green pipette, 200 µL, ZAF 101-5. Pipette tip ~425 pieces suitable to both white and green pipette, ZAF 101-6. Reaction tubes made of clear polycarbonate, 400 pcs, ZAF 101-7. Stoppers to reaction tubes, made of blue plastic, 400 pcs, ZAF 101-8. Instruction for use, ZAF 101-9.

## TEMPERATURE

Simple Simon<sup>®</sup> PT analyses PT in the temperature range 17-45°C.

## SAMPLE MATERIALS

The Simple Simon<sup>®</sup> PT perform PT analysis on 10µL of citrate anticoagulated plasma, citrate anticoagulated blood or native (capillary) blood.

## INSTRUCTION FOR USE

**Preparations, short instruction- for a more detailed version see text above “preparations”:**

1. Reconstitute PT-reagent, ZAF 101-2, by addition of 4 mL, one vial, buffer, ZAF 101-3, (temperature 2-8°C) into reagent vial. Turn the reagent vial upside down several times, at least 30 seconds, check that all dried material is completely dissolved.
2. The reagent is ready to use within 15 minutes from reconstitution. Within time range 1-3 hours from reconstitution the reaction tubes containing 200µL reagent should be placed in a refrigerator. Reagent is dispensed in reaction tubes, ZAF 101-7, 200µL in each. Dispensing is performed by pipetting 200µL using green pipette, ZAF 101-5 and a connected yellow pipette tip, ZAF 101-6. Tubes are sealed with blue stopper, ZAF 101-8. Store reagent in reaction tubes sealed by blue stoppers in a refrigerator Reconstituted reagent can be stored refrigerated (2-8°C) for three weeks, or maximum two (2) days in room temperature (17-26°C).
3. In connection to analysis, place a suitable number of reaction tubes containing reagent, in the aluminium plate holes on the backside of the reader. Tubes should be placed for at least 15 minutes to reach temperature equilibration before use. To check correct state use the control ZAP.

## Performing test:

1. Push button to activate the reader, ZAF 101-1. Reader shows the latest test results, running lot number and number of tests left. Check that lot-numbers of reader and components agree.
2. Push button, reader performs an “autocheck”. If check is accepted the message “+200µL reagent” shows on the display. If check not is accepted this can be due to temperature out of range, high background light of low background light (occlusion of light beam).
3. Place a reaction tube in measuring position- tube is attached by a bayonet joint and wait until countdown is finished. During that period reader check reagent criteria and looks for exaggerated turbidity or transparency. When moving reaction tube from the aluminium block into measuring position, the tube should be held in the stopper. Avoid touching the lower parts of the tube since heating from fingers could alter temperature equilibration. If reagent criteria are met, “10 µL sample & mix” flashes on the display. This message is necessary to continuation of analysis.
4. Place a pipette tip, ZAF 101-6, on the white pipette, ZAF 101-4. Secure the tip by a turning movement. When sample is sucked up in the tip, the air should be removed first by pushing the pipette piston using the thumb. Place pipette tip beneath the surface of the sample and suck it up with a gentle movement of thumb. Do not dispose of tip air content holding tip below sample surface. Do not repeat pipette piston movements below sample surface. This can result in incorrect volumes. When taking blood from a fingertip, the pipette tip should be held slightly angled towards skin surface to avoid blocking the tip.
5. Wipe tip with a piece of adsorbing paper to remove excess of sample, avoid touching the opening of tip causing removal of sample from tip.
6. Place pipette tip, containing 10 µL sample, just below reagent surface in the reaction tube which is placed in the measuring position of the reader. The start of the clotting reaction is automatically registered. Rinse pipette tip immediately by moving pipette piston up and down approximately five (5) times. Continue directly mixing of sample and reagent by rotating pipette tip in reaction tube. Movements are registered by a detector function and shown in percentage on the display. The minimum limit of acceptance is 90%. When maximum time span of mixing procedure is reached, Simple Simon demands “pipette out” on the display. Empty pipette tip and remove it from the reaction tube. The display shows “use cover” and operator puts the cover over the measuring position.
7. The display shows continuous INR calculations, progressing in real time. Reader registers time when clotting occurs, that is the clotting time.
8. PT analysis is complete when clotting has occurred. Display shows “clot” simultaneously as a picture of clotting action develops during eight (8) seconds. . Repeated black staples indicate that coagulation really has occurred. If detector has recognized a correct clotting pattern, it is accepted and “OK” shows. A clot picture lacking staples or only having a few staples indicates that clot detection is disturbed by something. Detector can not confirm clotting pattern and shows “error” on the display.

9. Display shows "remove cup". Remove the reaction tube. If sample is plasma, EVF<10%, "plasma INR" shows. If sample is blood "native INR" shows first. Another push of button shows "cit INR" which is the INR of a citrate anticoagulated blood sample.
10. Push button and EVF of the sample is shown. The determination of EVF is correct and precise enough to adjust INR values. The EVF value is not intended for laboratory diagnostic use.
11. Inactivate reader by pushing button down for two (2) seconds. Inactivation of reader can be performed at any stage of analysis. The inactivation is necessary before starting a new analysis. To minimize power consumption, reader automatically inactivates if no activity is recorded within ten (10) minutes.
12. Make an estimate if the test result is reasonable. If result seem unexpectedly low or high or can be regarded as atypical for the patient or is outside therapeutic limits, operator should repeat the test to secure that right test result is delivered. Duplicate results that differ more than 10% should be checked.
13. Perform a "reality check". The "reality check" means to lift the reaction tube out of measuring position and inspect whether content is liquid or solid. For example if reader shows INR >8, lift reaction tube and inspect. If sample is solid, clotting has occurred, the result may be incorrect. Same procedure is recommended if INR<0.7 and test result is expected to be in the therapeutic range. If sample is liquid, clotting has not occurred, the result may be incorrect. Repeat test to secure correct value.

#### Special considerations:

- To achieve correct test results, it is important that reagent and reader are temperature equilibrated. Allow reagent equilibration for a minimum of 15 minutes with placed reaction tubes in aluminium plate.
- Avoid touching reaction tubes with fingers.
- A thorough mix of sample and reagent is important to test results. Use the whole amount of time dedicated to mixing.

#### LIMITATIONS

It is not possible to perform PT analyses outside the temperature range 17-45°C. Avoid direct sunlight.

#### INTERPRETATION OF RESULTS

PT results are expressed in INR, International Normalized Ratio. INR is the quotient between sample and normal raised to the ISI- value of the procedure (ref 1).

#### REFERENCE VALUES

##### Expected values.

Normal range; INR 0,92-1,20 (70-130%). Therapeutic range in thrombosis prophylax; INR 2-3 (15-25%), ref 2.

#### PRECISION

Simple Simon<sup>®</sup> PT determines PT with equal precision as methods used at hospital laboratories.

#### STANDARDIZATION

Simple Simon<sup>®</sup> PT is delivered precalibrated. The product is calibrated indirectly to authentic plasma samples analyzed with international reference method using reference thromboplastin RBT/05 at the coagulation laboratory in Leiden. INR values are checked on hospital laboratories calibrated with Scandinavian calibrators delivered by EQUALIS ( External quality assurance in laboratory medicine in Sweden). Calibration procedure follows recommendations issued in "Certificate of analysis of calibrators and control materials intended for PT analysis (INR) according to Owren" (EQUALIS Version 2.0).

#### CONTROL MATERIALS

Control material to Simple Simon<sup>®</sup> PT may comprise of lyophilized control plasmas, comparing plasmas or blood controls. As an internal quality control Zafena recommends the use of Zafena Abnormal Plasma, ZAP, which is included in the product. ZAP has INR values in within the therapeutic range.

#### References

1. Besselaar A M H P van den. 1991. The significance of the International Normalized Ratio (INR) for oral anticoagulation therapy. JIFCC 3; 146-53.
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3. Lindahl TL et al. INR calibration of Owren-type prothrombin time based on the relationship between PT% and INR utilizing normal plasma samples. Thromb Haemost. 2004 Jun; 91(6):1223-31.
4. Ranby M. Coagulation tests at ambient temperature. Patent applicant ZAFENA AB. International Application Number PCT/SE2004/000910, International Publication Number WO 2004/111656.
5. Ranby M. Hematocrit and analyte concentration determination. Patent applicant ZAFENA AB International Application Number PCT/SE2004/001798.