1. INTENDED USE
The kit has been designed for the quantitative determination of Tissue Polypeptide Antigen (TPA) in human serum. The method can be used for samples over the range of 0-6000 IU/ml. The test has to be performed on the Maglumi fully auto analyzer (including Maglumi 1000, Maglumi 2000, Maglumi 2000 plus).

2. SUMMARY AND EXPLANATION OF THE TEST
Tissue polypeptide antigen (TPA) is a protein antigen identified immunologically by Bjerklund and Bjorklund (1957) in tumors using horse sera raised originally against the insoluble residues remaining after successive extractions of pooled human tumors. TPA antibodies can be raised using material from a single tumor, or from a mixture of different carcinomas, or using material from HeLa cells. TPA antigenicity can be identified in sera, in body fluids and in tissue extracts using radioimmunoassay, and kits are available commercially. In the last decade TPA has been studied extensively in serological testing as a general tumor-associated antigen even though the current literature raises some questions as to the validity of this claim. Thus, for instance TPA can be isolated from normal human placenta and using immunofluorescence microscopy TPA has been clearly demonstrated in certain epithelial cells in normal human tissues. TPA is related to keratins 8, 18 and 19 which are typical of simple epithelia and are also found in certain carcinomas.

TPA, as a marker was found only in tumor cells and suggest that currently available monoclonal antibodies specific for certain keratins could substitute for polyclonal TPA antibodies in certain applications.

3. PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay; Use an anti-TPA monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control, ABEI Label, FITC Label and magnetic microbeads coated with sheep anti-FITC are mixed thoroughly and incubated at 37°C, forming a sandwich. Then enter to a strong magnet field for separation lasting 36 seconds, cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of TPA present in controls or samples.

4. KIT COMPONENTS
4.1 Material supplies
Reagent Integral for 100 determinations
Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaNO₃, coated with sheep anti-FITC polyclonal antibody. 2.5ml
Calibrator low 3.0ml
Calibrator high 3.0ml
ABEI label: anti-TPA monoclonal antibody labeled ABEI, containing BSA, 0.2%NaNO₃. 10.5ml
FITC label: anti-TPA monoclonal antibody labeled FITC, containing BSA, 0.2%NaNO₃. 10.5ml

All reagents are provided ready-to-use.

4.2 Preparation of the Reagent Integral
Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!). Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 mins. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

4.3 Storage of the Reagents Integral
* Do not interchange magnetic microbeads from different lots

4.4 KIT CONTENTS

Accessories required but not provided
Maglumi Reaction module
Maglumi Starter kit 1+2
Maglumi Light check
Maglumi Wash /System Liquid
keep on using the Reagent Integral provided that the controls are
found within the expected ranges.

- Keep up-right for storage.
- Keep away from direct sunlight.

5. Origin of Calibrators
Calibrators in the Reagent Kit are from PROGEN.

6. Calibration
6.1 2 point recalibration
Via the measurement of calibrators, the predefined master curve is adjusted
(recalibrated) to a new, instrument-specific measurement level with each
recalibration.

6.2 Frequency of Recalibration
- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every 2 weeks and/or each time a new Integral is used (recommendation).
- After each servicing of the Maglumi Fully Auto analyzer.
- If controls are beyond the expected range.

7. Sample Collection, Material and Storage
- Collect samples using standard procedures.
- Sample material serum.
- Store at 2-8°C: 24 hours.
- For longer storage periods: freeze to below -20°C.
- Avoid repeated freezing and thawing cycles.
- Stored samples should be thoroughly mixed prior to use (Vortex mixer).
- Vacuum tubes
  - (a) Blank tubes are recommended type for collecting samples.
  - (b) If plasma sample is needed, EDTA tube is conformed has no
    effect on the results RLUs.
  - (c) Lipase Sin Sodium tube is found to increase the sample RLU and
    cause test results deviation.
- (d) Please ask SNIBE for advice if special additive must be used in
  the sample blood.

8. Interfering Substances
No interference with test results is seen by concentrations of bilirubin <
0.125mg/ml, haemoglobin <500mg/dl or triglycerides <12.5mg/ml.

9. WARNING AND PRECAUTIONS FOR USERS
- For use in IN-VITRO diagnostic procedures only.
- Do not interchange reagents from different lots. Do not use kit
  components beyond their labeled expiry date.
- All samples, biological reagents and materials used in the assay must
  be considered potentially able to transmit infectious agents. They
  should therefore be disposed of in accordance with the prevailing
  regulations and guidelines of the agencies holding jurisdiction over
  the laboratory, and the regulations of each country. Disposable
  materials must be incinerated; liquid waste must be decontaminated
  with sodium hypochlorite at a final concentration of 5% for at least half
  an hour. Any materials to be reused must be autoclaved using an
  overnight approach (USP 24,2000, p.2143). A minimum of one hour at
  121°C is usually considered adequate, though the users must check
  the effectiveness of their decontamination cycle by initially validating it
  and routinely using biological indicators.
- The calibrators in this kit are prepared from bovine serum products.
  However, because no test method can offer complete assurance that
  HIV, Hepatitis B Virus or other infectious agents are absent, these
  reagents should be considered a potential biohazard and handled
  with the same precautions as applied to any serum or plasma
  specimen.

10. Test Procedure
To ensure proper test performance, strictly adhere to the operating
instructions of the Maglumi Fully Auto analyzer. Each test parameter is
identified via a RFID tag on the Reagent Integral. For further information
please refer to the Maglumi Fully Auto Operator’s Manual.

11. Quality Control
- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control.

12. Results
12.1 Calculation of Results
The analyzer automatically calculates the TPA concentration in each
sample by means of a calibration curve which is generated by a 2-point
calibration master curve procedure. The results are expressed in IU/ml.
For further information please refer to the Maglumi Fully Auto Operator’s
Manual.

12.2 Interpretation of Results
- Reference values: <75IU/ml.
- Results may differ between laboratories due to variations in
  population and test method. Each laboratory should establish its own
  reference range.

13. Limitations of the procedure
13.1 A skilful technique and strict adherence to the instructions are necessary to
obtain reliable results.
- Procedure directions must be followed exactly and careful technique must be
  used to obtain valid results. Any modification of the procedure is likely to alter
  the results.
- Bacterial contamination or repeated freeze-thaw cycles may affect the test
  results.

13.2 HAMA
Patient samples containing human anti-mouse antibodies (HAMA) may give
falsely elevated or decreased values. Although HAMA-neutralising agents
are added, extremely high HAMA serum concentrations may occasionally
influence results.

13.3 High-Dose Hook
No high-dose hook effect was seen for TPA concentrations up to 100,000 IU/ml.

14. Performance Characteristics
14.1 Accuracy
Consider calibrator high of known concentration as a sample, dilute it by 1:2
ratio with diluent, and measure its diluted concentration for 10 times. Then
calculate the recovery of measured concentration and expected
concentration. The recovery should be within 90%-110%.

14.2 Precision
Intra-assay coefficient of variation was evaluated on Calibrator High
repeatedly measured 10 times in the same assay, calculating their coefficient
of variation, the results should ≤10%.

14.3 Sensitivity
The sensitivity is defined as the concentration of TPA equivalent to the mean
RLU of 20 replicates of the zero standard plus two standard deviations corresponding
to the concentration from the standard curve. The sensitivity is
typically less than 12.5IU/ml.

14.4 Specificity
The specificity of the TPA assay system was assessed by measuring the
apparent response of the assay to various potentially cross reactive analytes.
When CA211=1000IU/ml, the detection results of TPA <10IU/ml;
When CA724=1000IU/ml, the detection results of TPA <10IU/ml;
When CA242=1000IU/ml, the detection results of TPA <10IU/ml;

14.5 Linearity
Conduct a logarithmic transform to the RLU value and concentration value of
6 standards. After a double logarithmic fitting, the absolute value of its
linearity should exceed 0.9800.

15. References
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