Human PMN Elastase ELISA

Cat. No.: RD191021100R

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CONTENTS:

Use only the actual version of Product Data Sheet enclosed with the kit!

1. Intended Use

The RD191021100R Human PMN Elastase ELISA is a sandwich enzyme immunoassay for the quantitative measurement of the complex of human PMN elastase and α1-proteinase inhibitor (α1-PI) in plasma.
It is intended for research use only.

2. Storage, Expiration

Store the kit at 2 – 8°C. Storage conditions for aliquots of Standards, Quality Controls and samples are – 20°C. Shelf life of components is 30 days after opening or until the expiration date.

3. Summary

The human organism reacts with an inflammatory response to attacks of invading pathogens (micro-organisms and viruses) or damaged tissue (after accidents or surgery). Polymorphonuclear (PMN) granulocytes play an important role as primary defence cells in this inflammatory reaction. Different bloodstream mediators (cytokines, leukotrienes, complement factors, bacterial endotoxins, clotting and fibrinolysis factors) attract and stimulate these cells to phagocytize and destroy not naturally occurring agents.
PMN granulocytes use proteinases to digest these agents and tissue debris. One of these proteinases is PMN elastase which is localised in the azurophilic granules of the polymorphonuclear granulocytes. During phagocytosis of foreign substances these enzymes are also partially excreted into the extracellular surrounding, where the activity of PMN elastase is regulated by inhibitors (esp. the α1-proteinase inhibitor, α1-PI). An overwhelming release of PMN elastase, however, can exceed the inhibitory potential of the α1-proteinase inhibitor. Thus, enzymatically active PMN elastase, together with simultaneously produced oxidants (O_2-radicals, H_2O_2, OH-radicals), can cause local tissue injury.
Due to the bloodstream and lymphatic system, however, α1-PI is delivered subsequently and eventually able to form a complex with all excreted elastase. Therefore, the concentration of the PMN elastase/ α1-PI complex correlates with the released PMN elastase and can be used as a measure for the activity of granulocytes during an inflammatory response.
Primarily, determinations of PMN elastase find its application in observation of the course of trauma, shock and sepsis. Further indications are the areas of hemodialysis, infections by obstetrics, joint diseases, effusions of sport injuries, intestinal affection, pancreatitis, cystic fibrosis and male adnex affections.

4. Principle of the Test

The test kit is a solid phase enzyme immunometric assay (ELISA) in the microplate format, designed for the quantitative measurement of the complex of human PMN elastase and α1 proteinase inhibitor (α1-PI) in plasma. The microplate is coated with a first polyclonal antibody (egg yolk) against human PMN elastase (antigen).
Calibrators, controls and patient samples are pipetted into the antibody coated microplate. During a 60 minutes incubation present antigens in the sample bind to the antibodies fixed on the inner surface of the wells. Non-reactive sample components are removed by a washing step. Afterwards, a second polyclonal antibody against α1-PI, which is labeled with horseradish peroxidase, is added. During a 60 minutes incubation, the PMN elastase/ α1-PI complex bound to the first antibody is specifically recognized by the enzyme labeled antibodies, and a sandwich complex is formed. An excess of enzyme conjugate is washed out.
A chromogenic substrate, TMB (3,3',5,5'-Tetra-Methyl-Benzidine), is added. During a 20 minutes incubation, the substrate is converted to a colored endproduct (blue) by the fixed enzyme. Enzyme reaction is stopped by dispensing hydrochloric acid as stop solution (change from blue to yellow). The color intensity is direct proportional to the concentration of PMN elastase present in the sample.
The optical density of the color solution is measured with a microplate reader at 450 nm. Bi-chromatic measurement with a 600 - 690 nm reference filter is recommended.

5. Precautions

- During handling of all kit reagents the following precautions should be taken:
  Do not eat, drink or smoke.
  Do not pipette by mouth, use safety pipettes.
  Wear disposable gloves and avoid contact with kit reagents, control and sample material.
- The test kit contains components of human origin, which were found negative for Hepatitis B surface antigen and HIV (Human Immunodeficiency Virus). Nevertheless, for products derived from human or animal source it cannot be completely guaranteed, that they don't contain the above mentioned, others and not yet known or not diagnosticable pathogens. Sample material of patients (for example serum or plasma) normally used in laboratory determinations are always classified as potentially infectious.
- The assay reagents contain against microbial growth preservation substances, avoid contact with skin and/ or mucous membranes.
- Avoid contact with the TMB (3,3',5,5'-Tetra-Methyl-Benzidine) substrate solution containing peroxide. If it comes into contact with skin, wash thoroughly with water. Avoid contact with any easily oxidized materials. Extreme temperature changes may cause spontaneous decay of the peroxide.

- Avoid the contact with the stop solution containing acid. By skin contact, wash thoroughly with water. All instrumentation employed to dispense the stop solution should be thoroughly cleaned after use.

6. Reagents Supplied

Cat. No.	*Kit Components*	*Quantity*
C081111	Microtiter Strips (coated with polyclonal antibodies against PMN elastase), sealed	96 wells
C083141	Master Calibrator (containing PMN elastase/ α1-PI complex), lyophilized	2 µg
C084161	Quality Control 1-Low	1 vial
C084261	Quality Control 2- High	1 vial
C082111	Conjugate Solution (rabbit polyclonal antibody anti-α1-PI complex, enzyme labelled), ready to use	16 ml
C085011	Dilution Buffer, ready to use	50 ml
C086021	Wash Solution Concentrate (10x)	75 ml
C087101	Substrate Solution (TMB), ready to use	2 x 11 ml
C088311	Stop Solution (2 M HCl), ready to use	11 ml
-	Instruction Manual + Certificate of Analysis	1 pc

7. Materials Required but Not Supplied

- Distilled water
- Test tubes for diluting samples
- Precision pipettes to deliver 10 – 2000 µl
- Multichannel pipette 50 – 200 µl
- Graduated cylinders for 100 and 1000 ml
- Microplate shaker operating at 500 – 700 rpm
- Microtitration plate washer (optional). Manual washing is possible but not preferable.
- Microplate reader capable for endpoint measurements at 450 nm (optional reference filter in the range of 600 – 690 nm)
- Software package facilitating data generation and analysis

8. Preparation of Reagents

Attention:

- Do not interchange components of different kit lots.
- All components need to be brought at room temperature (18 – 28°C) before use.
- All components of these test kits, supplied as concentrate should be diluted to their final concentration at least 30 minutes prior to use. Mix well, but prevent of foam formation.
- Use a disposable-tip micropipette to dispense plasma samples. Pipet directly to the bottom of the wells. Change the tip between samples, to avoid carryover contamination.

Master Calibrator:

Reconstitute Master Calibrator with **2 ml** of Dilution Buffer at least 30 minutes prior to use. The concentration in the stock solution is 1000 ng/ml. Prepare set of standards as follows:

Calibrator volume	*Dilution Buffer volume*	*Concentration (ng/ml)*
500 µl of stock	-----	1000
500 µl of stock	500 µl	500
500 µl of std. 500	500 µl	250
500 µl of std. 250	500 µl	125
500 µl of std. 125	500 µl	62.5
500 µl of std. 62.5	500 µl	31.3
500 µl of std. 31.3	500 µl	15.6
-----	500 µl	0

This set of standards is ready to use now and need not to be diluted prior to use!
Storage conditions for aliquots are – 20°C.
Shelf life: 30 days after opening.

Quality controls – # 1 (Low), # 2 (High):

Reconstitute each vial of Quality Control with **1 ml** of Dilution Buffer at least 30 minutes prior to use.
The Quality Controls are ready to use now and need not to be diluted prior to use!
Storage conditions for aliquots are – 20°C.
Shelf life: 30 days after opening.

Wash Solution Concentrate (10x):

Dilute 75 ml of Wash Solution Concentrate with 675 ml of deionized (distilled) water.
Working Wash Solution is stable 6 months at 2 – 8°C.

9. Preparation of Samples

For determination of PMN elastase EDTA or citrated plasma are the preferred sample matrixes. Exsudate, bronchoalveolar lavage fluid and cerebrospinal fluid can be used. Serum is not suitable, because during clotting PMN elastase can be released *in vitro*. Culture supernatants are as well not suitable; the reason is, that the assay detects only the PMN elastase/ α1-PI complex and α1-PI is normally not present in culture medium.
Predilute all samples 1:100 with Dilution Buffer. Therefore 10 µl of sample may be diluted with 990 µl of Dilution Buffer.
The patients need not to be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate plasma from the cells by centrifugation.
For longer storage samples should be stored frozen at – 20°C. To avoid repeated thawing and freezing the samples should be aliquoted.

10. Assay Procedure

1) Pipet 100 µl of Standards, Quality Controls and samples, preferably in duplicates, into the appropriate wells.
2) Incubate the plate at temperature 25 – 30°C for 1 hour, shaking at ca. 700 rpm on an orbital microplate shaker.
3) Wash the wells 4-times with Wash Solution (300 µl per well).
4) Pipet 150 µl of Conjugate Solution into each well.
5) Incubate the plate at temperature 25 – 30°C for 1 hour, shaking at ca. 700 rpm on an orbital microplate shaker.
6) Wash the wells 4-times with Wash Solution (300 µl per well). Remove as much Wash Solution as possible by beating the microplate carefully.
7) Pipet 200 µl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight.
8) Incubate the plate for 20 minutes at room temperature, no shaking!
9) Stop the colour development by adding 50 µl of Stop Solution into each well and mix carefully.
10) Determine the absorbance by reading the plate at 450 nm. Bi-chromatic measurement with a reference at 600 - 690 nm is recommended. The absorbance should be read within 5 minutes following step 9.

11. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of standards versus *log* of the known concentration (X) of standards, using the four-parameter function. Results are reported as concentration of PMN elastase (ng/ml) in samples.
Alternatively, the *logit log* function can be used to linearize the calibration curve (i.e. *logit* of absorbance (Y) is plotted versus *log* of the known concentration (X) of standards).

12. Limits of Assay

Patient samples expected to contain higher PMN elastase concentrations than the highest calibrator (1000 ng/ml) should be more diluted (e.g. 1:200) with the Sample Diluent and assayed. The additional dilution step has to be taken into account for the calculation of the results.

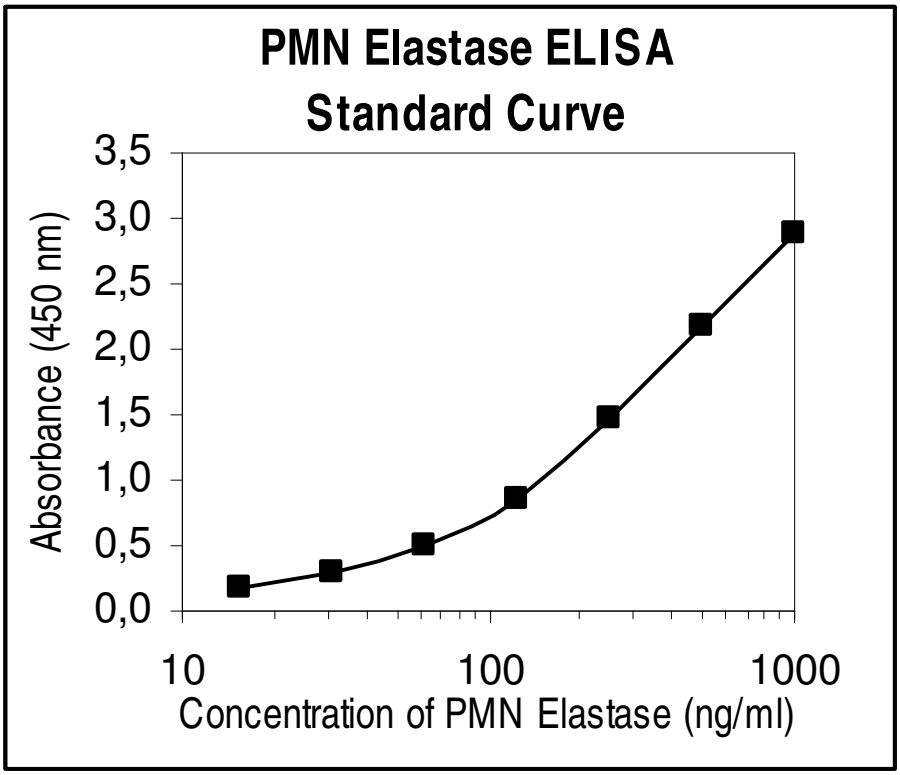
13. Performance Characteristics

Typical analytical data of BioVendor Human PMN Elastase ELISA are presented in this chapter. For actual Standard curve and Quality Controls values see the Certificate of Analysis.

- **Sensitivity**

The limit of detection (defined as human PMN Elastase concentration giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is defined as follows:
Analytical Limit of Detection is calculated from the real PMN Elastase values in wells and is 3ng/ml
Assay Sensitivity takes the dilution of samples into consideration and is calculated according to the formula:
Assay Sensitivity = Analytical Limit of Detection x sample dilution = 3ng/ml x 100 = 300ng/ml
**Dilution Buffer is pipetted into blank wells.*

PMN Elastase Standard Curve – example:



- **Specificity**

The BioVendor PMN Elastase test kit is specific for human PMN elastase only, respectively the PMN elastase/ α1-PI complex.

- **Precision**

Statistics for Coefficients of variation (CV) were calculated for each of three samples from the results of 10 determinations in a single run for Intra-Assay precision and the Inter-Assay precision was calculated from the results of 10 different runs of four samples.

Intra-assay

Sample No.	*Mean (ng/ml)*	*CV (%)*
1	80	5.2
2	241	4.7
3	358	4.6

Inter-assay (Run-to-Run, n=5), results are presented after calculation.

Sample No.	*Mean (ng/ml)*	*CV (%)*
1	128	5.7
2	216	6.4
3	346	4.4
4	681	5.7

- **Spiking Recovery**

Three spiking solutions were prepared (A – 922, B – 615 and C – 478 ng/ml). A 50 µl aliquot of each solution (A,B,C) was spiked into 950 µl aliquots of three different patient plasma samples, for a spiking ratio of 1 to 20, leaving the plasma matrix of the spiked samples relatively intact. All samples were then assayed.

Sample No.	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	23.2	-	-
1 + A	72.4	69.4	104
1 + B	59.3	54.1	109
1 + C	49.6	47.2	101
2	30.6	-	-
2 + A	73.4	76.7	96
2 + B	59.3	61.4	97
2 + C	56.8	54.4	104
3	61.7	-	-
3 + A	118.0	107.8	109
3 + B	100.8	92.5	109
3 + C	94.8	85.6	110

- **Linearity**

In dilution experiments sera with high PMN elastase concentrations were diluted with Dilution Buffer and assayed in the PMN elastase kit. The assay showed linearity over the fμll measuring range.

Sample No.	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	114.0	-	-
	1:2	57.9	55.7	103
	1:4	31.8	27.8	114
	1:8	14.6	13.9	105
2	-	135.6	-	-
	1:2	66.5	67.8	98
	1:4	30.8	33.9	91
	1:8	18.8	16.9	111
3	-	255.0	-	-
	1:2	130.5	127.5	102
	1:4	61.0	63.8	96
	1:8	31.9	31.9	100
4	-	540.1	-	-
	1:2	246.2	270.0	92
	1:4	119.9	135.0	89
	1:8	61.7	67.5	91
5	-	641.8	-	-
	1:2	281.4	320.9	88
	1:4	149.7	160.4	93
	1:8	69.9	80.2	87
6	-	909.5	-	-
	1:2	444.5	454.7	98
	1:4	208.1	227.4	92
	1:8	100.4	113.7	88

- **Effects of Bilirubin and Hemolysis**

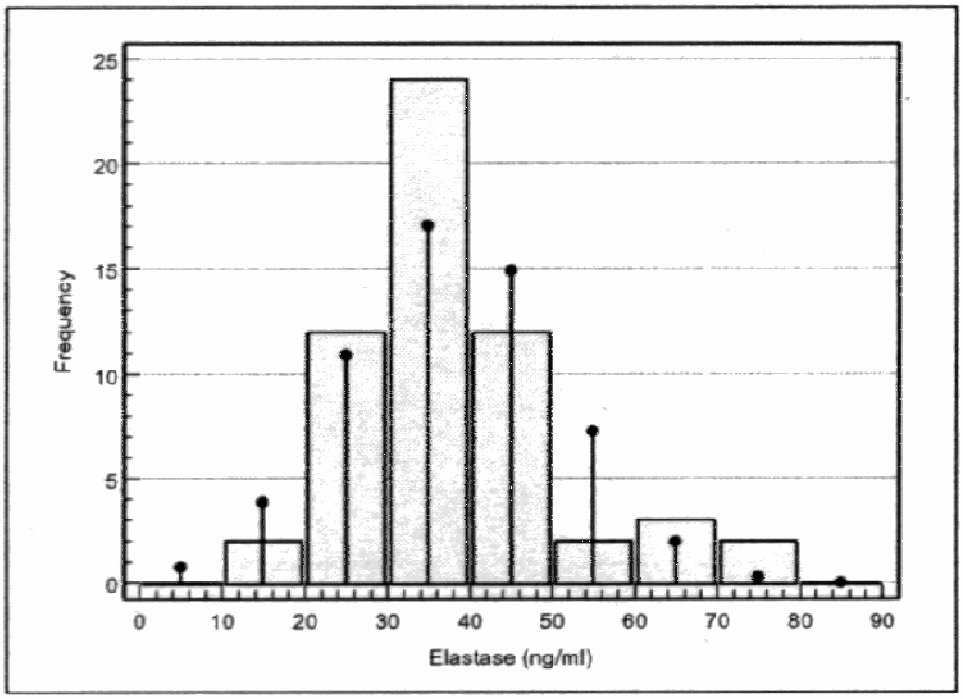
To simulate moderate and severe icterus, four samples were spiked with 100 and 200 milligrams of bilirubin per liter. All samples were assayed, both spiked and unspiked, with the following results (ng/ml). The results show that severe icterus (bilirubin up to 200 mg/L) has no clinically significant effect on the BioVendor PMN Elastase procedure. Samples with hemolysis normally show no effect on the BioVendor PMN Elastase procedure. In single cases hemolysis can lead to an increase due to the decay of granulocytes *in vitro*.

Sample No.	*Unspiked (ng/ml)*	*+ 100 mg/l Bilirubin*	*+ 200 mg/l Bilirubin*
1	100	106	104
2	249	245	261
3	572	575	534
4	903	964	910

- **Normal Values**

In a normal range study with plasma samples from healthy blood donors (n = 57) the following ranges have been established with the BioVendor Human PMN Elastase ELISA..

Distribution of PMN elastase in citrated plasma of healthy donors:

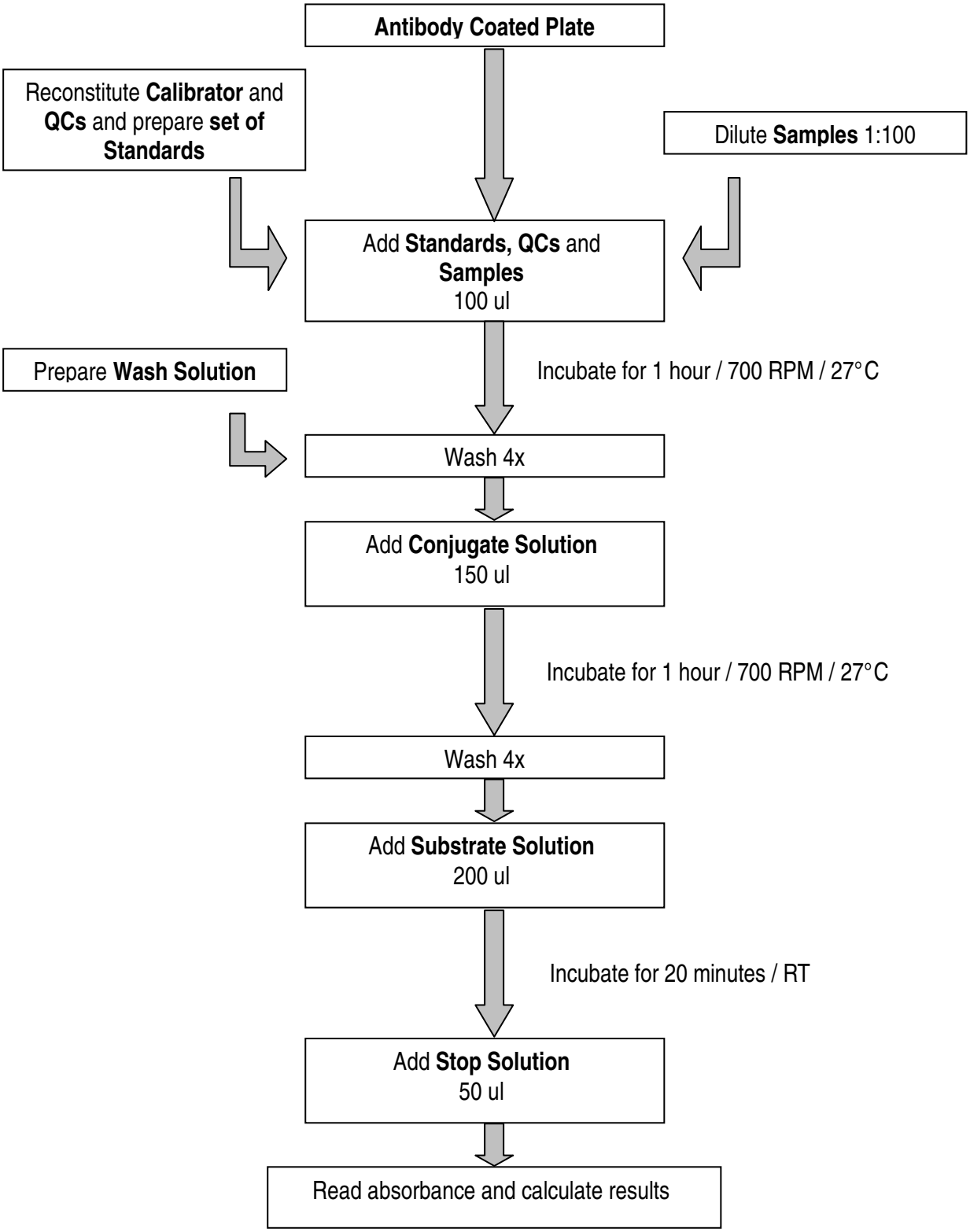


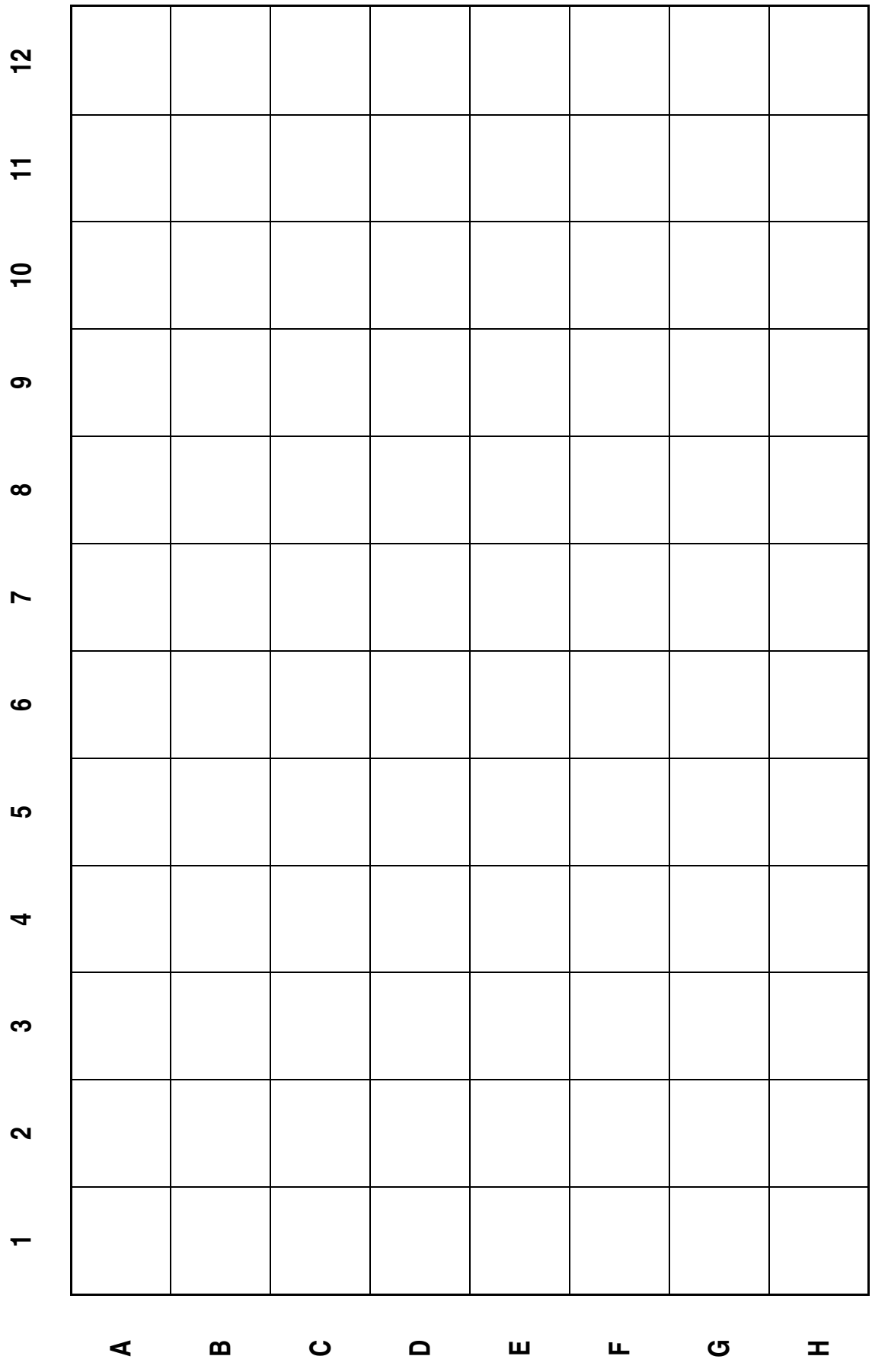
Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended to establish its own normal and pathological ranges of plasma PMN elastase for each laboratory.

14. References

- Jochum M., Machleidt, W., Neuhof, H., and Fritz, H. Proteinases. In: Schlag G, Redl H (eds), Pathophysiology of Shock, Sepsis, and Organ Failure. Springer-Verlag Berlin Heidelberg, 1993: 46-60.
- Jochum M., Machleidt, W., and Fritz, H. Proteolytic Enzyme Systems. In: Schlag G, Redl H (eds), Pathophysiology of Shock, Sepsis, and Organ Failure. Springer-Verlag Berlin Heidelberg, 1993: 531-548.
- Elastase In: Thomas, L. (Hrsg.) Labor und Diagnose Die Medizinische Verlagsgesellschaft, 4. Auflage, 1992: 795 – 801
- Elastase, Elastase-α1-Proteinase Inhibitor Complex In: Friedman, R.B., Young, D.S (Eds.) Effects of Disease on Clinical Laboratory Tests AACC Press, Washington, 3rd Edition, 1997: 3-161
- Reinhardt, A., Haidl, G., and Schill, W-B. Granulocyte elastase indicates silent male genital tract inflammation and appropriate antiinflammatory treatment. Andrologia 29, 1996: 187 – 192

Assay Procedure Summary

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5
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Notes: