PAPP-A [^{125}I] IRMA kit

(REF: RK-4CT)

The ^{125}I-PAPP-A IRMA system provides direct quantitative *in vitro* determination of pregnancy-associated plasma protein A in human serum, in the range of 0-45 µg/ml. Each kit contains material sufficient for 100 determinations, permitting the construction of one standard curve and the assay of 42 unknowns in duplicate.

Introduction

Pregnancy-associated plasma protein A is a large placenta-derived glycoprotein. The biological function of PAPP-A is not completely known, it has been shown its proteolytic and immuno-regulating action through cleavage of insulin-like growth factor binding protein 4.

PAPP-A maternal serum concentration rises during pregnancy. Measurement of PAPP-A, in conjunction with other parameters (free ßhCG concentration, fetal ultrasound, maternal age) can be used in the risk assessment of Down's syndrome during the first trimester of pregnancy. Mostly, values under 0.5 MOM (multiple of median) are considered pathological, however, isolated PAPP-A data have no diagnostic value by itself. For screening use, a computerized (parameter-weighted) interpretation of results is necessary with validated software. Each laboratory should determine its own reference (MOM) values.

Principle of method

This technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.

The ^{125}I labelled signal-antibody binds to an epitope of the PAPP-A molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During 2-hours incubation period with shaking, immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amounts of PAPP-A, the unknown concentration of PAPP-A in patient samples can be determined.

Contents of the kit

1. 1 bottle TRACER (21 ml), ready to use, containing about 740 kBq ^{125}I-anti-PAPP-A and capture anti-PAPP-A antibody in buffer with red dye and 0.1 % NaN_3.

2. 6 vials STANDARD (6 x 0.4 ml), ready to use, containing (S1-S6): 0, 0.45, 1.35, 4.5, 13.5, 45 µg/ml (1 IU/l = 4.5 µg/ml) in serum with 0.1% Kathon CG.

3. 2 vials of CONTROL SERUM (2 x 0.4 ml), ready to use, containing 0.1% Kathon CG. The concentration of the control serum is specified in the quality certificate enclosed.

4. 2 boxes COATED TUBE, ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

5. 1 bottle WASH BUFFER CONCENTRATE (20 ml), containing 0.1% NaN_3. See *Preparation of reagents.*

Quality certificate
Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (10, 200 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, absorbent tissue, gamma counter.

Recommended tools and equipment

Repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-ml pipette).

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Avoid repeated freezing-thawing cycles of samples. Hemolyzed and lipemic specimens may give false values and should not be used.

Preparation of reagents, storage

Add the wash buffer concentrate (20 ml) to 700 ml distilled water to obtain 720 ml wash solution. After dilution, store at 2-8°C until expiry date of the kit.

Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date of the kit. The actual expiry date is given on the package label and in the quality certificate.

CAUTION!

Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Label coated tubes in duplicate for each standard (S1-S6), control serum and sample.
2. Homogenize all reagents and samples by gentle mixing to avoid foaming.
3. Pipette 10 µl of standards, control and samples into the properly labeled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
4. Pipette 200 µl of tracer into each tube.
5. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
6. Incubate tubes for 2 hours, shaking at room temperature.
7. Add 2.0 ml of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
8. Return the tube-rack to an upright position, and repeat step-7 two more times.
9. Count each tube for at least 60 seconds in a gamma counter.
10. Calculate the PAPP-A concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**10**		
Control			**10**	
Sample				**10**
Tracer	**200**	**200**	**200**	**200**
Shake for 2 hours at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (CPM) for each pair of assay tubes.

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{2\text{-}6} / C / M_x\,(cpm) - S_1\,(cpm)}{T(cpm)} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of PAPP-A.

Determine the PAPP-A concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing, logit-log or spline fittings can be used. Automated data processing systems are also available.

Table 2. Typical assay data

Tubes	Count cpm	Mean cpm	B/T%
T	324642 330422	327532	-
S1	73 101	87	0.03
S2	2564 2627	2564	0.76
S3	7583 7386	7485	2.26
S4	25131 24176	24654	7.50
S5	71400 69580	70490	21.55
S6	178872 180902	179887	54.90
C1	35452 37484	36468	11.11
C2	68212 64829	66520	20.28

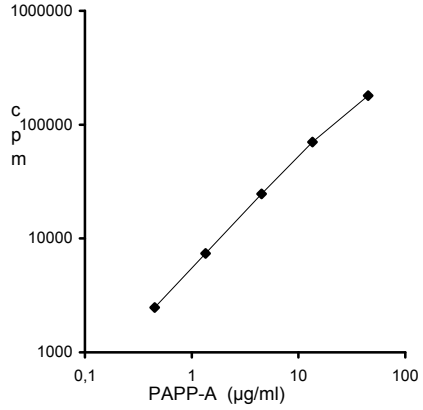

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Figure 1: A typical standard curve
(*Do not use to calculate unknown samples)*

Characterization of assay

Typical assay parameters

Bmax/T > 40%
NSB/T < 0.1%

Sensitivity

The analytical sensitivity is 0.0143 µg/ml, obtained by assaying 15 replicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the sum of the mean cpm and its double standard deviation.

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amounts of PAPP-A. The average per cent recovery for five serum pools spiked with PAPP-A at four levels was: 99.4 ± 3.3 % (mean ± SD).

Dilution test (linearity)

Five samples were measured in a series of dilution with zero-standard. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

Y = 1.047 x - 0.08 R = 0.9995 n = 25

Hook effect

The KIT has no "high-dose hook" effect with PAPP-A levels up to 800 µg/ml. Samples expected to have concentrations greater than the highest standard should be diluted with the zero standard and reassayed.

Precision and reproducibility

Seven patient samples were assayed in 15 replicates to determine intra-assay precision. To determine inter-assay precision seven patient samples were measured in duplicates in 22 independent assays using different kit batches. Values obtained are shown below.

Intra-assay		Inter-assay	
Mean (µg/ml)	CV %	Mean (µg/ml)	CV %
6.57	6.20	6.75	2.80
12.31	1.68	12.74	2.73
1.91	4.07	1.82	3.07
4.11	4.69	4.04	2.98
7.04	4.12	6.96	3.84
15.06	4.51	15.53	3.82
27.47	3.49	27.43	2.88

Expected Values

Pregnancy weeks	1MOM (µg/ml)	N
11	9.5	100
12	13.5	100
13	23.1	58

It is recommended that each laboratory determine a reference range for its own patient population. The results of this assay should be used in conjunction with other pertinent clinical information.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Addition of wash buffer.** For the addition of wash buffer the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Limitations

Without linked and validated software this kit is NOT intended to be used for the risk evaluation of trisomy 21!

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precaution

Radioactivity
This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard
Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1) and Hepatitis B surface Antigen (HBsAg).
Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.

Chemical hazard
Some components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 41 mg.
Some components contain Kathon CG as an antimicrobial agent. The total Kathon CG present in each pack is 3.2 mg.

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	WASHB	Wash buffer
IVD	In vitro diagnostic medical device	8°C 2	Temperature limitation Store between 2-8°C
	Manufacturer		Radioactive Material
REF	Catalogue number		

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