MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE

CENTRE DE DEVELOPPEMENT DES TECHNIQUES NUCLEAIRES

ALGERIA – REPORT

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« TUMOR MARKERS KITS DEVELOPEMNT FOR USE IN RADIOIMMUNOMETRIC ASSAYS »

FIRST MEETING OF THE CO-ORDINATED RESEARCH PROGRAMME.

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SOMMAIRE

I.INTRODUCTION

The immunoassays such as RIA and IRMA are now widely used through the world for the quantitation of a variety of substances in the biological fluid for their high sensibility and specificity which required simple equipments

These techniques are also very used in Algeria for an effective amelioration of public heath
The assays kits of RIA/IRMA of thyroid hormones are the most used, followed by peptidic hormones, steroids hormones and IRMA Tumor Markers (T.M) kits.

In spite of the important demand, of tumor markers kits for the diagnosis and follow up of cancers their use are always insuffisant due to the high cost

The AIEA has starting the project RAF/6/018 for the period 1997 – 2001 on the theme « The Consolidation Capability of Radioimmunoassays for Tumor Markers » that Algeria is joined, will allow certainly to cover in the first phase the request of reagents for IRMA « T M » retained in the programme of this project and in the second phase to give the possibility of our country to produce local reagents for IRMA assays « T.M »

The research contrat programme proposed by IAEA on the theme « The Developments of IRMA Tumor Markers Kits » of prostate specific Antigen (PSA) and Tissue Polypeptide Specific Antigen (TPS) will allowed us to produce localy with best quality-price, the main reagents for PSA and TPS IRMA assays kits for diagnosis and follow up the prostate and breast cancers which are very spready in the country

This report include the following points

-Generalities on the use of tumor markers in Algeria
-Programme for the Development of the PSA IRMA assay

*Shedule of protocoles applied for each reagents
*Annual planning for assessing the programme activities
-Conclusion
-Annex The need of chemical and biological reagents for the project

II. GENERALITIES ON THE USE OF TUMOR MARKERS ASSAYS IN ALGERIA.

1- COMMONEST CANCERS IN THE COUNTRY

The commonest five cancers for male and female are represented as follow :

Male	Female
-Lung	-Breast
Stomach	-Col-Uterus
Nephretic tractus	-Colon-Rectum
-Colon-Rectum	-Ovarium
-Bladder	-Gall-bladder

-Annual incidence of cancers

-M = 74,4/100.000

-F = 77,3/100.000

2 EXISTING AVAILABLE FACILITIES AND MANPOWER IN THE COUNTRY :

2.1 Number of cancer treatment facilities

-Hospital with cancer traitement facilities : 5
-Radiotherapy center : 5
-Medical oncology department : 5

2 2 Number of oncologists currantly practicing

-Medical oncologists about : 10
-Radiation oncologists about : 20

2.3 Available facilities for follow-up cancers patients :

The available facilities for follow-up of cancers patients are indicated as follow :

-Clinical examination
- X-ray and other simple tests
- Ultra sound
- Bone scane
- LT/MRI
- Mamography.

N.B : These informations are obtained from the cancers register of Algiers 1995 and from Medical Oncology Department.

3 LABORATORIES USER :

The laboratories using the kits of IRMA Tumor Markers are eight (08)
These laboratories are divided mainly at the hospitals in the North of the Country
-In the Centre (Algiers) : 04
-In the West (Sidi-Bel-Abbès, Tlemcen) : 02
-In the East (Constantine) : 01

All of these laboratories have qualified staff and adequat materiels to carry out all the RIA/IRMA assays and others similar methods.

4 THE KITS OF THE MAIN TUMOR MARKERS USED IN THE COUNTRY

The number of T.M imported from 1990 to 1996 : the mean price and the number of patients treated are presented in the table-I.

Table I – Tumor Markers kits data imported from 1990 to 1996.

KITS	Number of Kits imported 1990 - 1996	Mean price/Kit (U.S $)	Number of patients treated by Kit	Total number of patients treated.	Cost of the Assay by patient (U S $)
HTG	184	400	40	7360	10
B HCG	58	392	-«-	2320	9,8
HTC	49	460	-«-	1960	11 4
C.E.A	45	360	-«-	1800	9
A.F.P.	12	240	-«-	480	6

The number of Tumor Markers kits (table-I) imported during the period 1990-1996 didn't reflect the real demand of the users laboratories

This perturbation of tumor markers kits imported for IRMA assays are due to the high cost of these reagents and to the actuel hard economical situation of the country

We notice on the table I. that the price of the assay per patient is about (8US $)

All the kits mentionned on the table-I are imported from CIS-Bio International (FRANCE)

The kits of PSA tumor marker are not imported during this period.

5 ESTIMATIONS OF TUMOR MARKERS NEEDED FOR THE YEAR 1998

The estimation of IRMA Tumor Markers kits for the year 1998 are shown on the table-2

Table-2 Needs of Tumor markers kits for the year 1998

KITS	Number of kits
H.C.G	74
C.E.A	68
A.F.P	57
P.S.A	44
H.C.T	42
Ca-19-9	39
Ca-125	37
Ca-15-3	31
H.T.G	29
P.A.P	16

N.B : Informations obtained from users laboratories

III. DEVELOPMENT PROGRAMME OF THE MAIN REAGENTS FOR PSA IRMA ASSAY :

III.1 MAIN STEPS OF EXPERIMENTAL PROTOCOL TO PREPARE THE TRACER 125 I – Mab -ANTI-PSA

1.1 : Radioiodination of Monoclonal antibody Anti-PSA

The preparation of radiolabelled of MAb-I-125 Anti-PSA with high purity and good specific activity can be carried out by two comparatives methods based on the use of iodination agents chloromine-T and N. Bromosuccinimid.
-The optimization of radioiodination parameters will be realized (reagents concentration, reaction time etc...).

1.2 Purification of the tracer :

The purification of the tracer from chemicals and radiochemicals impurities can be carried out by column chromatography over Sephadex –G-25 gel or Sephacryl S-300, superfine with appropriate buffer.

1.3. Evaluation and standardization of the tracer :

The evaluation of MAb-I-125- Anti-PSA can be carried out by the estimation of the parameters mentionned as follow :

- Radiochemical purity (P.R.C) using physico-chemicals methods (i.e : Electrophoresis)
- Immunological activity : Estimation of the ratio of $\frac{\text{std max}}{T} \times 100$ and $\frac{\text{std}_0}{T} \times 100$ of differents purified fractions.

- Determination of specific activity
- Study of the stability of standardized and non standardized tracer under differents storage conditions (+4, -20°C) over time and possibility of lyophilization
- Standardization of the tracer by dilution with appropriate buffer to obtain a volumic concentration of 350 000 CPM/ml

1.4 Comparative study with commercial kit of P.S.A.

1.5 Choice of the best radioiodination agents between chloramine –T and N-Bromosuccinimid.

III-2 MAIN STEPS OF EXPERIMENTAL PROTOCOL FOR PREPARATION OF P.S.A STANDARDS

2.1 Preparation of PSA standards :

The preparation of PSA standards with differents concentrations are mentionned below

(O, 1.4,7,17,5,35,70,175 and 350 ug/L) can be carried out by pure form of imported antigen for PSA diluted in the appropriate matrice (i e humain serum), according to the protocol provided by the research agreement (RA) holders

2.2 Calibration of P.S.A standards :

-The prepared standards of P.S.A will be calibrated against references prepations (studie of parallelisme between the two standards curves), with determination of the dose at 20, 50, and 90% binding..
-The determination of the recuperation test
-The determination of the dilution test.

III-3 : MAIN STEPS OF EXPERIMENTAL PROTOCOL FOR THE PRODUCTION OF ANTISERUM ANTI-PSA.

3.1 Production of the antiserum anti-PSA :

The production of the antiserum anti-PSA can be carried out by injection of appropriate immunogen of PSA emulsified in Freund's adjuvant into experimental animals (rabbits) according to the protocole provided by the R.A Holder or that retained during the meeting.

3.2 Characterization of Antiserum anti-PSA :

For estimating the quality of the antisera produced, the following parameters will be assessed

- Titer estimation,
- Sensitivity
- Specificity studies.

These parameters will be with studied with double antibody PSA (imported RIA Kit)

3,3 Purification of antiserum :

-The best antisera will be purified by precipitation methods(ammonium sulfate or caprylic acid)
-Determination of the concentration of antiserum
-Another purification chromatography methods can be used
-The purified antisera will be used for coating solid phase (imported beads, or magnetisable particles)

III- 4 MAIN STEPS OF EXPERIMENTAL PROTOCOL FOR THE DEVELOPMENT OF THE SOLID PHASE :

4.1 : Choice of separation techniques for PSA IRMA assay :

The choice of solid phase method will be retained during the meeting.
We propose two solids phases : polystyrene beads and magnetisable particles.

4.2 Coating of pure antiserum anti-PSA to polystyrene beads or magnetisable particles :

The coating of pure anti-PSA (imported and localy prepared) to polystyrene beads or magnetisable particles can be carried out.

4.3 Optimization of coating :

To estimate the quality of coating the following parameters will be setting up

-temperature
-PH
-Time of reaction
-Concentration of the reagents

4.4. Optimization of separation system (Free and Bound).

The parameters which will be studied are
-PH
-Time of reaction (incubation)
-Temperature
-Washing, cycle of washing.

4.5 Studies of stability over period of time under differents conditions of storage .

III.5 MAINS STEPS OF EXPERIMENTAL PROTOCOL OF THE DESIGN OF PSA IRMA ASSAYS

The optimization assays and the validation of the kits for the PSA IRMA assay constitued with the reagents prepared locally are presented as follow :

5.1 assay optimization :

- Kinetic of antigen – antibody reaction
- Optimization of incubation conditions
- optimization of antibody concentrations

5.2 Validation of IRMA PSA assay :

- Estimation of range and sensitivity
-recovery test
-paralleliom test
-effect of protein concentration
-intra and inter assay coefficient of variation (reproductibility of the assay)
-comparative studies with commercial kits
-clinical analysis with normal patients serums

IV. ANNUAL SCHEDULE OF THE RESEARCH PROGRAMME

The annual schedule activities programme for development of the main reagents PSA IRMA assay are presented on the table-3.

Table-3 : Annual Schedule of research programme (1997/1998)

	MONTHS											
Activities	D	J	F	M	A	M	J	J	S	O	N	D
1. Providing the primary materiel from outside sources	X	X										
2. Production of PSA tracer :radioiodination purification and evaluation of radiolabelled Mab-I 125-Anti-PSA			X		X		X		X		X	
3. Preparation and evaluation of PSA standards .preparation of Qc materiel			X	X	X	X						
4. Production and caracterization of polyclonal antibody anti-PSA for solid phase preparation . purification of PSA antiserum.				X	X	X	X	X				
5. Preparation and evaluation of solid phase using imported bead and pure anti-PSA				X	X	X	X	X				
6 Preparation and evaluation of solid phase using imported bead and localy produced anti-PSA								X	X	X	X	
7 Assay design										X	X	X

V. CONCLUSION :

The realization and the succesful of this project will be efficient by an effective contribution of the differents involved parts.

The proposed programme for the development of the main reagents which constitute the PSA/IRAM kits is charged enough to be performed in a year. This programme should be studied very well during this meeting.

The progress of the activities depend on providing needs of imported reagents mentionned in annex of this programme.

Table-4 : NEEDS OF CHEMICALS AND BIOLOGICALS REAGENTS

Produits	Fournisseur	References	Quantité	Utilisation
1. Na_2H P04	SIGMA	S 7907	1 Kg	Buffer solution
2. NaH_2 P04. H_20	-« -	S 9638	1 Kg	-« -
3. Nacl	-« -	S 7653	1 Kg	-« -
4. Sodium Azide	-« -	S8032	100gr	Add. In Buffer
5. EDTA (Na_2EDTA-$2H_20$)	-« -	E 1644	500gr	-« -
6. Triton X-100	-« -	T 9284	500ml	-« -
7. Bovine Serum Albumin	-« -	A 3059	10x10g	-« -
8. Bovine gamma globuline	-« -	G7516	30x10 gr	-« -
9. Pure Antigen of P S A	VIA NETRIA OR WHO			Standards, Qc and Immunization
10. Pure Antiserum Anti-PSA (IgG)	-«-			Preparation of solid phase separation systeme
11. Monoclonal Antibody Anti-PSA	-«-		2x1mg	Preparation of Mab-3-125 anti-PSA.
12. Caprylic Acid	SIGMA		10x1gr	Purification of antiserum
13. Ammonium Sulfate			1 kgs	
14. Polyotyrene beads	VIA NETRIA OR WHO		2000 beads	Antibody coating
15. Glutaral de hyde	SIGMA	G 5882	2x10ml	For coupling Antibody
16. 1 Ethy -3 (3-Dimethy Aminopropyl) carbodimide (EDAC)	-« -	E 1769	5x1gr	

SUITE Table- 4.

19. Iodine - 125	Nordion			For Iodination (tracer)
20. N- Bromosuccinimid	Sigma	25 grs	B 9252	Labelling
Commercial kit of PSA -PSA beads -PSA standards (1ml) plus QC - I-125 –Anti-PSA - Tracer (370kbq)	NETRIA OR ODERS SOURCES	2000 4 sets 5 vials		Comparatives studies Of PSA reagents prepared localy.