IVD

CE

Cyclosporine direct RIA

KIPB3679

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History

Summary of change :

Previous Version : 131104/1	**Current Version :** 200224/1
No point **6. GHS Hazard classification**	Addition of point **6. GHS hazard Classification**
11. LIMITATION OF THE METHOD **Assay Procedure**	**11. LIMITATION OF THE METHOD** **Assay Procedure** Let all the reagents come to room temperature.
Old Diasource logo	New Diasource logo
No IVD symbol	IVD symbol added
LOT : 131104/1	Version: 200224/1
PI number	No PI number
No manufacturer symbol	Manufacturer symbol added

Cyclosporine direct RIA

en

For the In Vitro Determination of Cyclosporine in Human Whole Blood

KIPB3679

IN VITRO DIAGNOSTIC

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PRINCIPLE OF THE ASSAY

The specific assay of cyclosporine A is a radioimmunological competition assay. Unknown samples, controls, and calibrators are incubated in monoclonal antibody-coated tubes with ^{125}I-labeled cyclosporine tracer. After incubation, the liquid content of tubes is aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are determined by interpolation from a calibration curve.

REAGENTS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit label, if stored at 2-8°C. Expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take into account.

Storage conditions for reagents after reconstitution or dilution are indicated in paragraph Assay Procedure.

Specific anti-cyclosporine monoclonal antibody coated tubes: 2 x 50 tubes (ready-to-use)

Ag	125I

^{125}I-labeled cyclosporine: 2 x 28 mL vials (ready-to-use)
One vial contains 125 kBq, at the date of manufacture, of 125I-labeled cyclosporine in buffer containing <20% Ethanol (see § Precautions), bovine serum albumin and a dye.

CAL	N

Calibrators: 6 x 0.5 mL vials (ready-to-use)
The calibrator vials contain from 0 to approximately 2500 ng/mL of cyclosporine in bovine serum with sodium azide (<0.1 %; see § Precautions). The exact concentration is indicated on each vial label. Calibrators are verified to an internal reference standard.

CONTROL	N

Control serum: 2 vials (lyophilised)
The vials contain cyclosporine lyophilized in bovine serum. The expected values are indicated on the vial label.

MATERIAL PROVIDED BUT NOT REQUESTED

In addition to standard laboratory equipment, the following items are required:

- precision micropipet (20 µL).
- adjustable dispenser (500 µL).
- vortex-type mixer.
- horizontal or orbital shaker.
- aspiration system.
- gamma counter set for ^{125}I.

PRECAUTIONS

1 General remarks:

- The vials with calibrators and controls should be opened as short time as possible to avoid evaporation.
- Do not mix the reagents from kits of different lots.
- A calibration curve must be established with each assay.
- It is recommended to perform the assay in duplicate.
- Each tube must be used only once

2 Basic rules of radiation safety

The purchase, possession, utilization, and transfer of radioactive material is subject to the regulations of the country of use.

Adherence to the basic rules of radiation safety should provide adequate protection:

- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipeting of radioactive solutions by mouth.
- Avoid all contact with radioactive materials by using gloves and laboratory overalls.
- All manipulation of radioactive substances should be done in an appropriate place, distant from corridors and other busy places.
- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.
- Radioactive waste should be handled according to the rules established in the country of use.
- This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.

3. Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper or brass to form explosive metal azides. Dispose of the reagents by flushing with large amounts of water through the plumbing system.

4 Human whole blood

All whole blood samples should be handled as if capable of transmitting hepatitis or AIDS and waste should be discarded according to the country rules.

5 <20% Ethanol

R 10 Flammable

6 GHS hazard classification

Tracer WARNING
H226 Flammable liquid and vapour.
P210 Keep away from heat, hot surfaces, and sparks. No smoking.
P242 Use non-sparking tools.
P280 Wear protective gloves, protective clothing and eye/face protection.
P303+P361+P353 IF ON SKIN (or hair): Rinse skin with water.
P370+P378 In case of re: Use water spray for extinction.
P501 Dispose of contents/container in accordance with local/national regulations
Ethyl Alcohol 10 – 20%

SPECIMEN COLLECTION, PROCESSING AND STORAGE

- Collect whole blood samples into tubes containing EDTA. The use of citrate or heparin is not recommended. Blood with EDTA should be thoroughly mixed immediately after sampling to avoid erroneous result caused by local coagulation.
- Whole blood samples may be stored at 2-8°C, if the assay is to be performed within a week. For longer storage keep frozen (<-20°C) for up to 2 months, after aliquoting so as to avoid repeated freezing and thawing.
- Cooled and, particularly, frozen samples has to be brought up to room temperature and mixed gently for 15-20 minutes on hematological rocker to be sufficiently homogenized. It is also possible to lay the tightly capped tubes with samples on your laboratory shaker and let them roll about. Too short mixing may not be sufficient and it may result in erroneous result, especially in frozen samples.

ASSAY PROCEDURE

1 Preparation and storage of reagents
Let all the reagents come to room temperature.

1.1 Reconstitution of control samples
The content of the vials is reconstituted with the volume of distilled water indicated on the label. Wait for 10 min following reconstitution and mix gently to avoid foaming before dispensing. Store the reconstituted solutions at 2-8°C for one week or frozen at < -18°C for a longer time, until the expiry date of the kit.

2 Assay procedure (see table next page)

RESULTS

Results are obtained from the calibration curve by interpolation. The curve serves for the determination of cyclosporine concentrations in samples assayed at the same time as the calibrators.

1 Calibration curve
The results in the package insert were calculated using a logit-log curve fit (weighted cubic regression) with B/T (%) or B/B_0 (%) on vertical axis and the cyclosporine concentrations of calibrators on the horizontal axis (ng/mL). Other data reduction methods may give slightly different results.

Total activity: 83 705 cpm				
Calibrators	**Cyclosporine (ng/mL)**	**cpm (n=3)**	**B/T (%)**	**B/B_0 (%)**
0	0	60 455	72.2	100.0
1	36	54 096	64.6	89.5
2	95	44 538	53.2	73.7
3	210	36 044	43.1	59.6
4	520	22 478	26.9	37.2
5	2 235	9 295	11.1	15.4

(Example of calibration curve, do not use for calculation)

2 Samples
For each sample or control, locate the B/T (%) or the B/B_0 (%) value on the vertical axis and read off the corresponding cyclosporine concentration in ng/mL on the horizontal axis.

QUALITY CONTROL

Good laboratory practices imply that control samples must be used regularly to ensure the quality of the results obtained. These samples must be processed exactly the same way as the assay samples, and it is recommended to analyze their results using appropriate statistical methods.
In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following E-mail address: tech.support@diasource.be.

EXPECTED VALUES

It is difficult to establish therapeutic ranges for cyclosporine because there are no simple parameters for the assessment of its immunosuppressive effect. Transplant centres have derived therapeutic ranges empirically on the bases of their own and published experience. These values are different for various graft types and for different analytic method, but they differ also between individual transplant centres for the same graft type and analytic method. The CsA therapeutic ranges also depend on the concomitant administration of other immunosuppressive medications.
A clinical evaluation was performed using extraction version of the assay (Cyclosporine specific RIA) in kidney, heart, liver and pancreas transplant patients treated with Cyclosporine. The trough blood values were measured post transplant up to 25 weeks. No significant event (rejection, nephrotoxicity or hepatotoxicity) was observed except of one slight rejection in liver transplant group.
Because of the very good correlation between the results of extraction version (Cyclosporine specific RIA kit) and this direct version, the findings of the evaluation may be applied for this Cyclosporine direct RIA kit as well.
Temporal CsA whole blood values in kidney, heart, liver and pancreas transplant patients measured in transplant centre by Cyclosporine specific RIA kit.

Post Operative Week	Kidney			Heart[1]			Liver[2]			Pancreas[3]		
	CsA (ng/mL)			CsA (ng/mL)			CsA (ng/mL)			CsA (ng/mL)		
	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.
0	10	165	83	5	142	78	7	186	38	8	283	96
1	9	217	63	6	209	96	7	178	56	7	222	93
2	6	322	69	5	357	121	5	247	91	7	218	82
2-4	5	327	64	9	388	63	5	211	57	8	211	74
4-7	8	301	90	7	349	112	5	179	63	8	212	36
7-10	5	213	39	5	322	62	4	158	22	3	173	106
10-15	5	260	64	6	309	94				6	182	57
15-25	8	204	51	4	299	30						

n = number of measured values

[1] Cyclosporine: 2.0 mg/kg/day to 5.8 mg/kg/day, Azathioprin or Mycophenolate mofetil and Prednisone were given

[2] Cyclosporine: 1.5 mg/kg/day to 9.8 mg/kg/day, Azathioprin or Prednisone were given

[3] Cyclosporine: 5.4 mg/kg/day to 7.1 mg/kg/day, Mycophenolate mofetil 2x1 g daily, Prednisone 20 mg daily decreasing gradually

PERFORMANCE CHARACTERISTICS

(For more details, see the data sheet "APPENDIX")

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

1.1 Analytical sensitivity: 1.61 ng/mL

1.2 Functional sensitivity: 32.8 ng/mL

2 Specificity
The antibody used in the immunoassay is specific for cyclosporine. Very low cross-reactivities were obtained with cyclosporine metabolites.
Several drugs were assayed at different levels and found not to interfere with the assay.

3 Precision

3.1 Intra-assay
Samples were assayed in 20 replicates in the same series. The coefficients of variation were found below or equal to 9.2 %.

3.2 Inter-assay
Samples were assayed in duplicate in 10 different series. Coefficients of variation were found below or equal to 7.3 %.

4 Accuracy

4.1 Recovery test
Whole blood samples were spiked with known quantities of Cyclosporine. The recovery percentages were obtained between 95.7 % and 120 %.

5 Measurement range (from analytical sensitivity to highest calibrator):
1.61 to approximately 2500 ng/mL.

11. LIMITATION OF THE METHOD

The non-respect of the instructions in this package insert may affect results significantly.
Do not use lipemic or icteric samples.
Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information.
For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays.
Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

ASSAY PROCEDURE

Let all the reagents come to room temperature.

Step 1 Additions *	Step 2 Incubation	Step 3 Counting
To antibody coated tubes, add successively: - 20 µL of calibrator, control or blood sample (mixed just before pipetting) and - 500 µL of tracer. Mix.	Incubate 60 minutes **at 18-25 °C** with shaking (> 280 rpm).	Aspirate carefully the contents of tubes (except the 2 tubes «total cpm»). Count activity (cpm) for 1 min.

* Add 500 µL of tracer to 2 additional tubes to obtain total cpm.

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