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免疫沉淀法测定乳酸脱氢酶同工酶 1 的研究

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摘要 应用免疫沉淀法测定了乳酸脱氢酶同工酶 1 (LD1). 血清与抗血清用量为 10∶1, 加入抗血清 5min 后再加入与血清量相等的饱和硫酸铵溶液. 离心后测定上清液中的 LD. 总酶活性在 618U/L 内线性关系良好. 二份标本批内精度 *CV* 值分别为 3.76%和 4.70%, 批间 *CV* 值为 7.00%. 免疫沉淀法与电泳法高度相关 ($n=22$, $r=0.976$). 72 例正常人 LD 参考值为 102.31±16.4U/L, LD1 为 23.65±4.39U/L, LD1/LD 为 23.12±3.85%. 免疫沉淀法的优点是特异性高, 操作简单, 可用于自动生化分析仪. 精确度高, 线性关系好, 测定范围宽, 是测定 LD1 的理想方法.

关键词 乳酸脱氢酶同工酶 1 (LD1), 免疫沉淀法, 抗 LD_m 抗体

在人体各种组织细胞中已发现五种乳酸脱氢酶 (LD, EC 1.1.1.27) 的同工酶, 分别为 LD1, LD2, LD3, LD4 和 LD5. 其中心肌中主要含 LD1. 肝细胞中主要含 LD5. 因而同工酶的测定有特殊的诊断意义. 测定同工酶的方法有电泳法、化学抑制法、酶切抑制法和免疫沉淀法. 电泳法可测定各种同工酶的相对含量. 结果可靠. 但需要特殊仪器, 操作复杂, 难以普遍应用. 其他方法目前均为测定 LD1. 化学抑制法和酶切抑制法国内已有报道[1-3]. 免疫沉淀法在国外早有报道[4-7], 但国内尚未见正式报道. 我们对免疫沉淀法测定 LD1 进行了方法学的研究. 我们认为本法特异性高, 操作简单, 精确度和线性关系好, 测定范围宽, 是一种理想的方法. 总结如下.

1 材料和方法

1.1 试剂 LD_m 抗血清和饱和硫酸铵溶液 (沉淀剂) 由北京医院检验科、奥斯邦公司提供. LD 试剂盒来自上海长征公司.

1.2 血标本 来自本院病人和体检人员. 取静脉血, 凝固后分离血清.

1.3 方法 a. 免疫沉淀: 取血清 200μl 加入抗血清 20μl (血清与抗血清为 10∶1) 混匀, 室温放置 5min. 加硫酸铵溶液 200μl (与血清量相等) 混匀, 室温再放置 5min, 3000r/min 离心 5min, 取上清液进行测定. b. 用 BT-2245ARCO 自动生化分析仪测定上清液中的 LD 含量即为 LD1. 参数如下:

方法: 速率法; 延迟时间: 30s; 波长:

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340nm；读出时间：30s；温度：30℃；试剂量：400μl；因数：2450；标本量：63μl（含血清30μl）.

2 结 果

2.1 测定条件的探讨

2.1.1 抗血清用量：同一份标本各取200μl加到7支试管中，分别加入1.25，2.5，5，10，15，20和25μl抗血清进行抑制. 5份标本测定结果见表1. 结果表明5—10μl抗血清能抑制标本中除LD1以外的所有其他LD同工酶. 我们选用20μl抗血清可保证有足够的抗体.

表1 不同剂量抗血清对测定值的影响

（U/L）

抗血清量/μl	1.25	2.5	5	10	15	20	25
标本1	96	86	80	80	80	78	74
标本2	270	238	221	212	210	212	210
标本3	100	89	85	87	91	85	80
标本4	217	183	165	168	170	175	168
标本5		192	167	162	168	174	153

2.1.2 抗血清作用时间：同一份标本各取200μl加到6支试管中，分别加入抗血清2，5，7，30，60和120min后加入饱和硫酸铵溶液. 5份标本测定结果见表2. 结果表明加入抗血清2min后抗原抗体已经完全结合. 120min内是稳定的. 我们选用5min作为抗原抗体作用时间.

表2 抑制时间对LD1测定值的影响

（U/L）

抑制时间/min	2	5	7	30	60	120
标本1	46	46	45	46	47	47
标本2	112	109	115	112		
标本3	136	136	136	136		
标本4	173	167	170	169	174	174
标本5	200	200	205	208	198	201

2.2 特异性

我们分别将原血清和抑制后的上清液做电泳观察抑制效果. 电泳扫描的结果见图1. 免疫沉淀后上清液中只剩下LD1，其他含LD_m亚基的同工酶全部结合成抗原抗体复合物被沉淀. 表明本法特异性非常高.

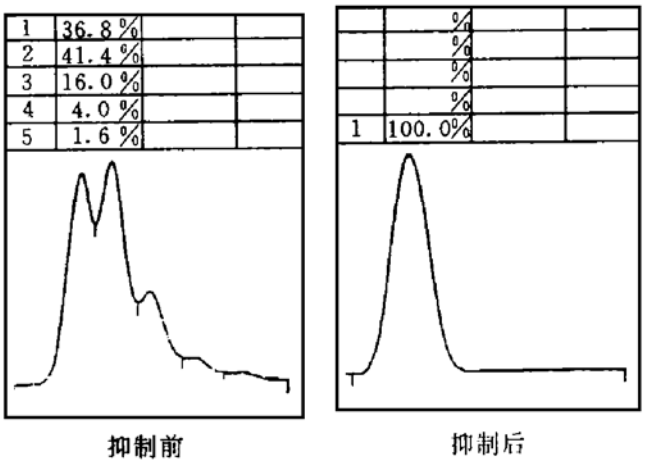

图1 免疫沉淀前后电泳扫描图

2.3 线性 选用LD总酶活性为618U/L的标本倍比稀释后测定LD1. 原血清，2，4，8，16和32倍稀释后测定LD1分别为370，179，95，49.7，25.2和12.4U/L. 线性关系如图2.

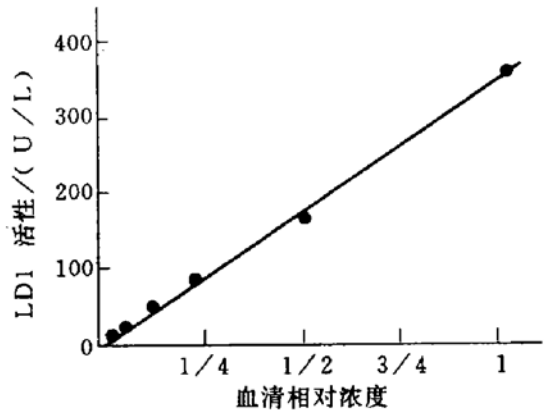

图2 线性关系

2.4 精确度

2.4.1 批内：每份标本同时测定10次. 2份标本测定结果如表3.

2.4.2 批间：同一份标本在5d内共测定10次. x = 56.3U/L，s = 3.98U/L，CV = 7.00%.

表 3 免疫沉淀法测 LD1 活性批内精度

标本	X/ (U·L^{-1})	S/ (U·L^{-1})	CV/%
1	79.8	3.0	3.76
2	50.9	2.4	4.70

2.5 免疫沉淀法和电泳法比较

22 例标本用免疫沉淀法和电泳法同时测定 LD1，并计算二者相关关系. 结果表明二者高度相关 (r=0.976，a=9.86，b=0.87)，二者相关图见图 3.

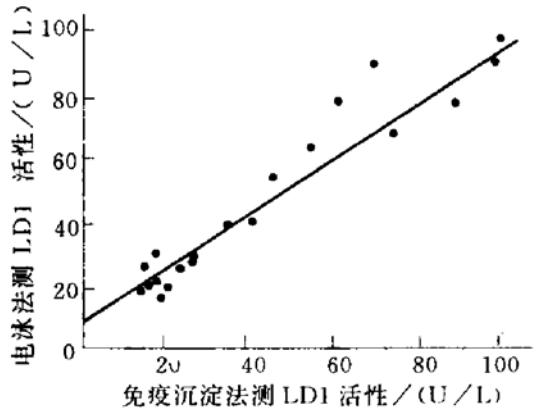

图 3 免疫沉淀法与电泳法测定 LD1 相关图

参考值：测定 72 例体检人员血清总 LD 和 LD1，LD 为 102.31±16.40U/L，LD1 为 23.65 ± 4.39U/L，LD1/LD 为 23.12 ± 3.85%. 未统计性别和年龄之间差异.

3 讨 论

乳酸脱氢酶的分子中含有 M 亚基和 H 亚基. 这两种亚基分子量相同，但结构和理化性质不同. LD 的各种同工酶都是由 M 亚基和/或 H 亚基组成的四聚体. 其中 LD1 由 4 个 H 亚基组成，LD5 由 4 个 M 亚基组成. LD2，LD3 和 LD4 分别由 H_3M，H_2M_2 和 HM_3 组成. 我们使用的抗血清为羊抗人 LD_m 亚基抗体. 当抗血清与血清标本混合后，抗体与含 M 亚基的 LD 分子结合成抗原抗体复合物. 加入沉淀剂后该复合物被沉淀下来，上清液中只剩下不含 M 亚基的 LD1. 因此用本法测定 LD1 较化学抑制法和酶切抑制法特异性高.

国外有人用免疫沉淀法测定 LD1 效果很好. 特异性和敏感性均较高，但他们使用的是双抗体法，即用第二抗体沉淀含 M 亚基的 LD 及其抗体的抗原-抗体复合物，由于第二抗体制备较复杂，难以推广普及. 而我们使用化学沉淀法，用硫酸铵代替第二抗体，一切试剂均为国产，价格便宜，实验结果与国外报道相近.

本法特异性高，操作简便，可用于自动生化分析仪，线性关系好，测定范围宽，另外还有一个优点是在操作中不需要严格控制温度和时间. 因而测定结果稳定，并且可大批量测定. 但化学抑制法和酶切抑制法都必须严格控制抑制时间，在实际应用中会受到很大限制，难以用自动生化分析仪大批量测定.

本法与电泳法比较二者高度相关. 但测定值较电泳法低. 而且我们发现 LD 值较低的标本，免疫沉淀法测定 LD1 值明显低于电泳法. 而 LD 过高的标本则免疫沉淀法测定 LD1 值高于电泳法. 可能是 LD 过高时电泳法不能反映各种同工酶比例. 有关这个问题有待进一步研究.

致谢 承蒙北京医院生化室周序开主任，李义龙和罗玲同志提供试剂并协助做电泳，特表示感谢.

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was modulated by the ratio of different nucleotides in reaction system. Through this binding, the interaction of VIP and it's receptor was regulated.

Key words VIP, photo-affinity, nucleotide

Study on Sensitivity Improvement of Dissociation Enhanced Lanthanide Fluoroimmunoassay. Zhao Qiren, Zhang Fuhua, Lu Jie, Lin Han. (*Institute of Radiation Medicine, Chinese Academy of Medical Sciences, Tianjin* 300192). *Prog. Biochem. Biophys.* (China), 1994: **21** (3): 255

Factors influencing the sensitivity, or the signal/noise ratio, of dissociation enhanced lanthanide fluoroimmunoassay (DELFIA) have been studied. The fluorescence responses and signal/noise ratios for different europium amount were shown to be changed with the volume of enhancement solution, and there was an optimum volume at a certain europium amount. The smallest europium amount leads to the smallest optimum volume. 20% of the net fluorescence intensity was increased by using tinfoil reflection layer. Effective washing and drying methods of microtitration strips decreased background fluorescence have been developed.

Key words time-resolved fluoroimmunoassay, dissociation enhanced lanthanide fluoroimmunoassay, sensitivity

Purification of Cuprozinc Superoxide Dismutase From Human Erythrocytes by Cu^{2+} Chelate Affinity Chromatography. Lu Xing, Chen Jizhong, Li Peifeng, Yang Suhong, Fang Yunzhong. (*Beijing Institute of Radiation Medicine, Beijing* 100850). *Prog. Biochem. Biophys.* (China), 1994; **21** (3): 259

Cuprozinc superoxide dismutase (CuZn-SOD) from human erythrocytes was purified by a procedure involving Cu^{2+} chelate affinity chromatography. It was shown in three experiments that the special chromatography held a number of important advantages for protein purification, such as a high rate of repeating performance and a large protein capacity. The purified enzyme, with a specific activity of 3073 U/mg protein, was tested for homogeneity by activity-stained and SDS gel electrophoresis. Accompanied by the study, a simple and efficient method was worked out for assessing the homogeneity of CuZn-SOD using its ratio of the absorbence at 260nm to that at 280nm.

Key words cuprozinc superoxide dismutase, metals-chelate affinity chromatography, human erythrocyte, purification

Determination of Isoenzyme 1 of Lactate Dehydrogenase by an Immunoprecipitation Method. Wu Xiyun, Yue Xiuling, Chen Yan, Si Xuezhong, Wang Zhongquan. (*Clinic Laboratory, Beijing TianTan Hospital, Beijing* 100050). *Prog. Biochem. Biophys.* (China), 1994; **21** (3): 262

Isoenzyme 1 of lactate dehydrogenase (LD1) was measured by an immunoprecipitation method. The antibody to M subunit of LD was added to the patient's serum and incubated for 5 min, at room temperature. The ratio of serum to antibody was 10 : 1. After incubation, a saturated ammonium sulphate solution was added with the same volume as serum. Then, centrifuged to precipitate all M-containing isoenzyme (LD2—LD5) as insoluble antigen-antibody complex. Determined the residual activity of LD in supernatant fluid. The relationship Between the LD activity and absorbance was linear up to 618U/L. Within-run

coefficient of variation (*CV*s) of two samples were 3.8% and 4.7%. Day-to-day coefficient of variation (*CV*s) was 7.0%. The correlation of immunoprecipitation method and electrophoretic procedure were cinsistent ($n=22$, $r=0.976$). Reference values for total LD and LD1 were 102.3±16.4U/L and 23.7±4.4U/L respectively. The ratio of LD1 to total LD was 23.1±3.9%. The advantages of immunoprecipitation method were: high specificity. good precision and linearity. easy operation. Immunoprecipitation method was very suitable for measuring LD1 and could adaptable to the automatic analyzer.

Key words isoenzyme 1 of lactate dehydrogenase (LD1). immunoprecipitation method. anti-M antibody

New Semi-dry Technique in SDS PAGE. Guo Yaojun. Wen Tao. (*Institute of Biophysics, Academia Sinica. Beijing* 100101). *Prog. Biochem. Biophys.* (China). 1994: **21** (3): 265

Shorter running time. higher resolution. simpler operation without preparation of large amount of electrode buffer is the superior character of SDS PAGE semi-dry technique. It is more convenient with buffer soaked filter strips instead of electrode buffer with filter bridge or buffer gel strips.

Key words semi-dry technique. SDS PAGE. filter strips

A New Assay for Kinase Activity: The Determination of Protein Kinase Using Capillary Electrophoresis. Wan Qian. Chen Changzheng. Li Boliang. Xia Qichang. (*Shanghai Institute of Biochemistry, Academia Sinica. Shanghai* 200031). *Prog. Biochem. Biophys.* (China). 1994: **21** (3): 267

A new assay of protein kinase A using capillary electrophoresis has been established. It is a universal and useful method for kinase activity. The method based on principle that substrate (kemptide) and product (phosphorylated kemptide) are easily seperated by capillary electrophoresis and the enzyme activity can be calculated on the integrated area. A continous sampling technique which can analyse more than 10 samples in one run has also been developed. The new method is easy to operate and its accuracy and sensitivity are higher than that of conventional isotopic method.

Key words protein kinase A activity. capillary electrophoresis. phosphorylation

The Variation of Content in *Tetrahymena pyriformis* During Ageing. Wang Bing. Liu Biansheng. Xing Yiyin. (*Hubei Geriatric Institute. Wuhan* 430071). *Prog. Biochem. Biophys.* (China). 1994: **21** (3): 268

Digital Image Analysis is a new DNA content measuring method in cell nucleus. During the senile process of *Tetrahymena pyriformis*. change of DNA content in nucleus could be measured by this method. According to law of Beer-Larmbert. cell nucleus in different growth period showed change of nuclear DNA content using level of nuclear integral optical density. The method possess quick measuring speed. well repetition. simple operation and good results. The results showed: when *Tetrahymena pyriformis* began the logarithmic growth phase. the nuclear DNA content reached peak gradually. When cell ageing gradually. the times of cell division as well as DNA content would be gradually decreased.

Key words *Tetrahymena pyriformis*. DNA content. digital image analysis. ageing