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Brucellacapt、RBT、SAT、iELISA 四种血清学检测方法对 布鲁氏菌病检测价值的比较研究*

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摘要 目的:探讨布鲁氏菌病血清学试验(Brucellacapt)、虎红平板凝集试验(RBT)、试管凝集试验(SAT)、间接酶联免疫吸附试验(iELISA)四种血清学检测方法对布鲁氏菌病检测价值的比较研究。**方法:**收集近两年 110 例布鲁氏菌病疑似病例人员的静脉血分离得到血清后进行 Brucellacapt、RBT、SAT、iELISA 四种血清学检测,以卫生部制定的《布鲁氏菌病诊疗指南》中布鲁氏菌病确诊方法为诊断金标准,将检测结果与其确诊结果进行比较,评价比较各组血清检测方法对布鲁氏菌病的检测价值。**结果:**110 例疑似人员中确诊为布鲁氏菌病阳性 91 例、阴性 19 例。Brucellacapt 试验阳性 89 例、阴性 21 例;RBT 试验阳性 79 例、阴性 31 例;SAT 试验阳性 71 例、阴性 39 例;iELISA 试验阳性 82 例、阴性 28 例。Brucellacapt 试验的灵敏度、符合率、Kappa 值、ROC 曲线下面积均最大,iELISA 试验、RBT 试验、SAT 试验依次减小;iELISA 试验的 ROC 曲线下面积最大,Brucellacapt 试验次之,其次为 RBT 试验,SAT 试验的 ROC 曲线下面积最小。**结论:**Brucellacapt、RBT、SAT、iELISA 四种血清学检测方法对于布鲁氏菌病检测均有一定的检测价值,对于常规普通患者可采用 RBT 试验、SAT 试验进行检查,而对于疑似病例人员可采用灵敏度更高的 Brucellacapt 试验、iELISA 试验。

关键词:布鲁氏菌病;血清学试验;虎红平板凝集试验;试管凝集试验;间接酶联免疫吸附试验

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Comparison of Four Serological Detection Methods of Brucellacapt, RBT, SAT and iELISA in the Detection of Brucellosis*

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ABSTRACT Objective: To study the comparative study of four serological detection methods of brucellosis (Brucellacapt), tiger red flat agglutination test (RBT), test tube agglutination test (SAT) and indirect enzyme linked immunosorbent assay (iELISA) for the detection of brucellosis. **Methods:** Blood samples were collected from 110 suspected cases of brucellosis in the past two years, and then serological tests for Brucellacapt, RBT, SAT and iELISA were carried out in four sera. The diagnostic method of brucellosis in the guidelines for the diagnosis and treatment of brucellosis made by the Ministry of health was the diagnostic gold standard. The results were compared with the results of the diagnosis, and the value of serum detection methods for brucellosis was evaluated and compared. **Results:** Among the 110 suspected cases, 91 were brucellosis positive and 19 were negative. Brucellacapt test was positive 89 cases and negative 21 cases. RBT test was positive 79 cases and negative 31 cases. SAT test was positive 71 cases and negative 39 cases. iELISA test was positive 82 cases and negative 28 cases. The sensitivity, coincidence rate, Kappa value and the area under the ROC curve of Brucellacapt test were the largest. The iELISA test, the RBT test and the SAT test decrease in turn; the area under the ROC curve of the iELISA test was the largest, the Brucellacapt test was the next, the next was the RBT test, and the minimum area under the ROC curve of the SAT test was the smallest. **Conclusion:** The four serological testing methods of Brucellacapt, RBT, SAT and iELISA have certain value for detection of brucellosis. RBT test and SAT test can be used for routine ordinary patients. The sensitivity of Brucellacapt test and iELISA test can be used for patients with suspected cases.

Key words: Brucellosis; Brucellosis serology test; Tiger red agglutination test; Tube agglutination test; Indirect ELISA

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前言

布鲁氏菌病是一种感染了布鲁氏菌而引起的人畜共患的传染性疾病,患者发病后可表现出急性症状与慢性症状,但大多数症状不典型,诸如发热、多汗、乏力、关节炎、睾丸炎、失眠、食欲不振、上呼吸道感染等^[1-3]。若患者未及时确诊,则有可能延误患者的治疗时机,导致患者的病情进一步发展而引起临床恶性不良事件的发生^[4,5]。因此,尽早的诊断、确诊、治疗和疗效评估对于布鲁氏菌病患者的健康有着重要作用。临床通过血清免疫学检查结合病原学细菌分离实验确诊布鲁氏菌病,但是该方法操作复杂,检查内容较多,难以达到快速确诊的需求^[6,7]。近些年,随着血清学检测理论和技术的不断发展,其检测快速、高效、准确度高等优点逐渐被临床认可,在布鲁氏菌病的检测中也有着较高的认可度^[8]。常用的血清学检查方法有虎红平板凝集试验(Tiger red plate agglutination test, RBT)^[9]、试管凝集试验(Tube agglutination test, SAT)^[10]等,近几年布鲁氏菌病血清学试验(Brucellacapt)、间接酶联免疫吸附试验(Indirect enzyme-linked immunosorbent assay, iELISA)等较为新颖的检测方法逐步开始应用,但是对于其临床检测效果以及对布鲁氏菌病的检测灵敏度、特异度的研究报道仍然较少。为此,本研究以研究团队近两年收治的布鲁氏菌病疑似病例人员为研究对象,分别采用 Brucellacapt、RBT、SAT、iELISA 四种血清学检测方法进行检测,判断比较各种方法的检测价值。

1 资料与方法

1.1 样本资料

以 2016 年 1 月-2018 年 1 月期间 110 例布鲁氏菌病疑似病例为研究对象,其中男性 58 例、女性 52 例,年龄 20~78 岁,平均(42.39±12.97)岁。纳入标准^[11]:①疑似人员诊断检测前与携带布鲁氏菌的家禽家畜有过接触史,或生活在布鲁氏菌病流行区;②疑似人员有布鲁氏菌病的临床症状表现,包括发热、乏力、多汗、肌肉和关节疼痛等;③疑似人员精神状态正常,能够配合临床血清学实验检测;④疑似人员签署知情同意书。排除标准:①并发全身恶性肿瘤疾病者;②严重的心肝肾功能不全者;③未成年患儿、妊娠哺乳期妇女;④合并自身免疫性疾病者。研究方案经中国人民武装警察部队学院伦理学委员会批准。

1.2 检测方法

受试者收治后采集静脉血约 5 mL,加入到抗凝管中,采用 TGL-16 型高速离心机(上海安亭科学仪器厂)进行离心操作 10 min,离心速度为 5000 r/min,分离得到血清备用。

1.2.1 Brucellacapt 试验 采用 Brucellacapt 检测试剂盒(赛默飞世尔科技有限公司)进行血清 Brucellacapt 试验,当检测结果滴度 $\geq 1/320$ 时判断为阳性,即受试者感染布鲁氏菌,否则为阴性。

1.2.2 RBT 试验 采用布鲁氏菌虎红平板凝集抗原试剂盒(武汉默沙克生物科技有限公司)进行血清 RBT 试验,当受试者血清在 4 min 内出现肉眼可见凝集现象者即可判断为阳性,即受试者感染布鲁氏菌,无凝集现象则判断为阴性。

1.2.3 SAT 试验 采用布鲁氏菌试管凝集抗原试剂盒(武汉默沙克生物科技有限公司)进行血清 SAT 试验,当检测结果滴度 $\geq 1/100$ 即为阳性,否则为阴性。

1.2.4 iELISA 试验 采用 iELISA 试剂盒(深圳市科润达生物工程有限公司)进行血清 iELISA 试验,采用 LumiStation 1800Plus 型化学发光酶标仪(上海闪谱生物科技有限公司)进行检测,当测定结果大于截断值 0.5 为阳性,小于截断值 0.5 为阴性。

1.2.5 确诊方法 以卫生部制定的《布鲁氏菌病诊疗指南》^[12]中布鲁氏菌病确诊方法为诊断金标准,即疑似或临床诊断病例出现多项免疫学检查至少有 1 项为阳性并且受试者细菌学实验分离得到布鲁氏菌及可确诊为布鲁氏菌病^[6]。

1.3 评价方法

将检测结果与受试者的确诊结果进行比较,并计算各种检测方法的灵敏度、特异度、符合率,其中灵敏度=真阳性/(真阳性+假阴性)、特异度=真阴性/(真阴性+假阳性)、符合率=(真阳性+真阴性)/(真阳性+真阴性+假阳性+假阴性)。并比较各检测方法结果与金标准结果的一致性,检测方法一致性采用 Kappa 检验,Kappa 值为 0~1,值越大表明一致性越高,通过绘制 ROC 曲线计算各检测方法的 ROC 曲线下面积。

1.4 统计学方法

采用 SPSS 21.0 进行数据处理与分析,计量资料以($\bar{x} \pm s$)表示,组间比较采用 t 检验,计数资料以[n(%)]表示,组间比较采用 χ^2 检验,检测方法一致性采用 Kappa 检验,当 $P < 0.05$ 时差异有统计学意义。

2 结果

2.1 Brucellacapt、RBT、SAT、iELISA 检测结果

110 例疑似人员中确诊为布鲁氏菌病阳性 91 例、阴性 19 例。Brucellacapt 试验阳性 89 例、阴性 21 例;RBT 试验阳性 79 例、阴性 31 例;SAT 试验阳性 71 例、阴性 39 例;iELISA 试验阳性 82 例、阴性 28 例,见表 1~表 4。

表 1 Brucellacapt 试验与金标准诊断结果比较

Table 1 Comparison of diagnostic results between Brucellacapt test and gold standard

Brucellacapt	Gold standard results		Total
	Positive	Negative	
Positive	85	4	89
Negative	6	15	21
Total	91	19	110

表 2 RBT 试验与金标准诊断结果比较

Table 2 Comparison of diagnostic results between RBT test and gold standard

RBT	Gold standard results		Total
	Positive	Negative	
Positive	74	5	79
Negative	6	14	31
Total	91	19	110

表 3 SAT 试验与金标准诊断结果比较

Table 3 Comparison of diagnostic results between SAT test and gold standard

RBT	Gold standard results		Total
	Positive	Negative	
Positive	65	6	71
Negative	26	13	39
Total	91	19	110

表 4 iELISA 试验与金标准诊断结果比较

Table 4 Comparison of diagnostic results between iELISA test and gold standard

iELISA	Gold standard results		Total
	Positive	Negative	
Positive	79	3	82
Negative	12	16	28
Total	91	19	110

2.2 Brucellacapt、RBT、SAT、iELISA 检测评价结果

灵敏度、符合率、Kappa 值、ROC 曲线下面积规律表现为：
Brucellacapt 试验 > iELISA 试验 > RBT 试验 > SAT 试验，特异

度为：iELISA 试验 > Brucellacapt 试验 > RBT 试验 > SAT 试验，
见表 5，ROC 曲线见图 1。

表 5 Brucellacapt、RBT、SAT、iELISA 检测评价结果

Table 5 Test results of Brucellacapt, RBT, SAT and iELISA

Test method	Sensitivity(%)	Specificity(%)	Coincidence rate(%)	Kappa	ROC area under curve
Brucellacapt test	93.41(85/91)	78.95(15/19)	90.91(100/110)	0.771	0.896
RBT test	81.32(74/91)	73.68(14/19)	80.00(88/110)	0.641	0.720
SAT test	71.43(65/91)	68.42(13/19)	70.91(78/110)	0.582	0.691
iELISA test	86.81(79/91)	84.21(16/19)	86.36(95/110)	0.714	0.805

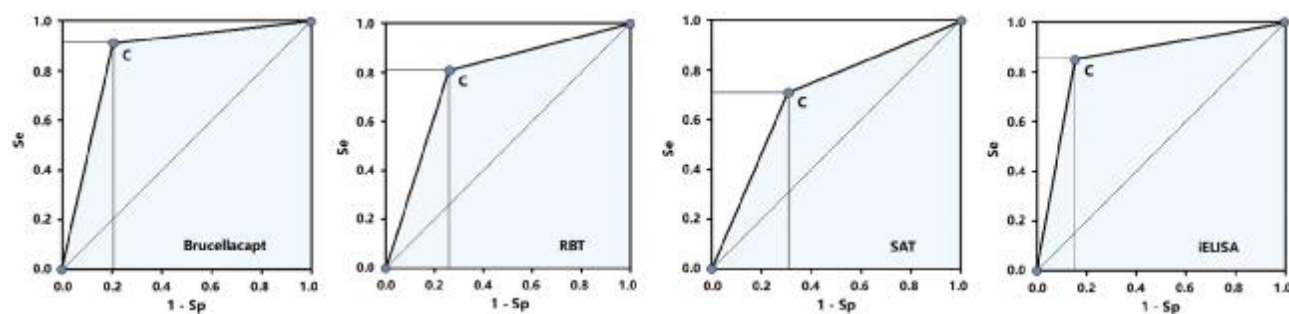


图 1 Brucellacapt、RBT、SAT、iELISA 试验方法的 ROC 曲线

Fig.1 ROC curves of Brucellacapt, RBT, SAT and iELISA test methods

3 讨论

布鲁氏菌病是一种人畜共患的高度传染性疾病,该病早期无特异性症状,到中后期病情迅速进展,患者的全身系统、骨骼系统、心肌等均有严重损伤,有强烈的致畸危害^[13-15]。因此,有必要对患者进行早期的诊断确诊,并尽早的开展相应的治疗以减轻布鲁氏菌对患者的危害^[16]。目前,在《布鲁氏菌病诊疗指南》中对布鲁氏菌病的诊断通常需要结合多种诊断方法结果进行综合判断,主要包括临床症状表现、实验室血象、血沉检测、免疫学检查和细菌学检查等手段,确诊的标准为疑似人员免疫学检查中 SAT、补体结合试验、布病抗 - 人免疫球蛋白试验中有至少一项是阳性,并且疑似人员细菌学检查分离得到布鲁氏菌病^[17,18]。金标准方法虽然能够准确做出诊断,但是诊断过程复杂、操作繁琐费时,不利于快速做出诊断。血清学检测方法具有快速、简便、高效等特点,已经广泛应用于布鲁氏菌病的检测和诊断,其中 SAT、RBT 试验已经纳入布鲁氏菌病的确诊方法中,并发挥重要作用^[19-21]。随着血清学检测技术的不断更新,Brucellacapt、iELISA 等新的检测方法也崭露头角,显示出了较高的诊断价值^[22-23]。

在本研究中,对疑似布鲁菌病疑似人员采用 Brucellacapt、RBT、SAT、iELISA 四种血清学方法进行检测,结果显示 Brucellacapt 试验的灵敏度、符合率、Kappa 值、ROC 曲线下面积均最大,iELISA 试验、RBT 试验、SAT 试验依次减小;iELISA 试验的 ROC 曲线下面积最大,Brucellacapt 试验次之,其次为 RBT 试验,SAT 试验的 ROC 曲线下面积最小。上述结果充分表明 Brucellacapt、RBT、SAT、iELISA 四种血清学方法在布鲁氏菌病的检测有重要价值,新的血清学检测技术 Brucellacapt、iELISA 试验与常规检查方法 RBT、SAT 相比有较高的灵敏度和特异度,其中灵敏度反映了该检测方法确定阳性患者的能力,特异度反映的是该检测方法发现阴性患者的能力。符合率即反映该方法确定真阳性患者和真阴性患者的能力,Kappa 值则可以体现该检测方法与金标准检测结果的一致性。ROC 曲线则是一种全面、有效、准确评价检测方法的指标,当曲线下面积越大表明该检测方法的真实度越高。Brucellacapt 试验是在双抗体夹心 ELISA 试验基础上发展得到的一种免疫捕获凝集试验,其检测标志为免疫球蛋白 IgG、IgM、IgA 抗体,同时也可检测出阴性抗体,从而做出准确诊断^[24-26]。iELISA 试验是用聚苯乙烯包被布鲁氏菌的脂多糖抗原进行检测,通过对布鲁氏菌的 IgG、IgM 抗体的识别,达到准确诊断的目的^[27,28]。Brucellacapt、iELISA 两种试验均具有操作简便,不需要特定的仪器设备,易于临床推广等优点,对于临床布鲁氏菌病的确诊提供重要的依据^[29,30]。

综上所述,Brucellacapt、RBT、SAT、iELISA 四种血清学检测方法对于布鲁氏菌病检测均有一定的检测价值,但是 Brucellacapt、iELISA 试验的灵敏度、特异度、一致性均要高于常规血清学检测方法,在该类疾病诊断中有重要应用价值。

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