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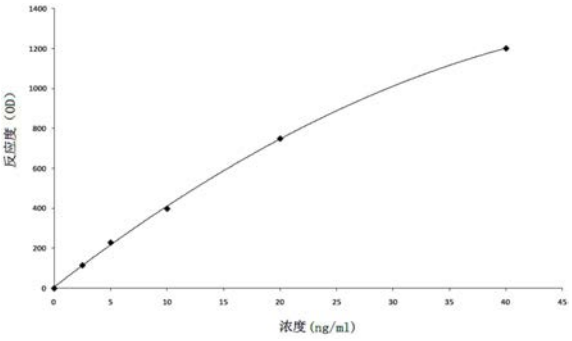
(54)发明名称

一种用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法

(57)摘要

本发明公开了一种用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，包括制备R2试剂的步骤：S1、提取幽门螺杆菌抗原(尿素酶)蛋白，将尿素酶基因克隆到pGEX-4T-1质粒中，通过大肠杆菌发酵表达，经过亲和层析纯化得到重组幽门螺杆菌抗原(尿素酶)；S2、制备抗幽门螺杆菌抗原(尿素酶)多克隆抗体；S3、制备包被抗幽门螺杆菌抗原(尿素酶)多克隆抗体的乳胶颗粒；S4、分散于缓冲溶液得到R2试剂。该方法是乳胶增强免疫比浊法，制得的试剂是一种可直接通过粪便检测幽门螺杆菌的溶液，仅需粪便样品即可完成检测，取样方便，对人体无安全隐患，检测效率高、稳定性好，特异性强，不易受干扰。



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1.一种用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，包括制备R2试剂的步骤：

S1、提取幽门螺杆菌抗原蛋白，将尿素酶基因克隆到质粒中，通过大肠杆菌发酵表达，得到重组幽门螺杆菌抗原；

S2、制备抗幽门螺杆菌抗原多克隆抗体；

S3、制备包被抗幽门螺杆菌抗原多克隆抗体的乳胶颗粒；

S4、将包被抗幽门螺杆菌抗原多克隆抗体的乳胶颗粒与含有0.1％-10％电解质、0.1％-10％稳定剂、0.1％-10％表面活性剂、0.1％-5％防腐剂和1-1000mmol/L的MES缓冲液混合，即得。

2.根据权利要求1所述的用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，所述步骤S1具体为，选择尿素酶做为幽门螺杆菌抗原，所述尿素酶包括UreA和UreB蛋白亚基，将UreA或UreB基因克隆到质粒中，通过大肠杆菌发酵表达以及亲和层析纯化，即得幽门螺杆菌抗原，所述质粒包括但不限于pGEX-4T-1质粒，所述质粒包含蛋白标签基因，所述蛋白标签包括但不限于GST蛋白。

3.根据权利要求2所述的用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，所述步骤S2包括：

S21、将幽门螺杆菌抗原用等量弗氏完全佐剂乳化；

S22、将乳化后的抗原进行动物皮下注射，进行免疫反应；

S23、取经过免疫的血清进行亲和层析处理后即得抗幽门螺杆菌抗原多克隆抗体。

4.根据权利要求3所述的用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，所述步骤S3包括如下步骤：

S31、将乳胶颗粒与缓冲溶液混合并加入表面活性剂，得到乳胶颗粒溶液，所述乳胶颗粒占所述乳胶颗粒溶液的质量百分比为0.01-10％；

S32、将所述抗幽门螺杆菌抗原多克隆抗体溶解于缓冲溶液得到抗体溶液，所述抗幽门螺杆菌抗原多克隆抗体占抗体溶液的质量百分比为0.01-10％；

S33、将所述乳胶颗粒溶液与所述抗体溶液混合后，加入1-(3-二甲氨基丙基)-3-乙基碳二亚胺盐酸盐，所述1-(3-二甲氨基丙基)-3-乙基碳二亚胺盐酸盐占混合溶液的质量百分比为0.01％-5％，室温下反应2-4小时，得到包被抗幽门螺杆菌抗原多克隆抗体的乳胶颗粒。

5.根据权利要求4所述的用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，还包括制备R1试剂的步骤，所述R1试剂包括0.1％-10％的电解质、0.1％-10％的稳定剂、0.1％-10％的表面活性剂、0.1％-5％的防腐剂，余量为浓度1-1000mmol/L的MES缓冲液。

6.根据权利要求5所述的用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，还包括制备校准品的步骤：以重组幽门螺杆菌抗原为母液，用牛血清蛋白溶液梯度稀释所述重组幽门螺杆菌抗原，即得不同浓度的校准品，所述牛血清蛋白溶液的浓度为1ng/ml-100g/l。

7.根据权利要求6所述的用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，所述R1试剂、R2试剂中的所述电解质包括但不限于氯化钠，所述稳定剂包括但不限

于甘露醇，所述表面活性剂包括但不限于吐温80，防腐剂包括但不限于叠氮钠。

8.根据权利要求7所述的用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，所述乳胶颗粒的粒径为50-500nm。

9.根据权利要求8所述的用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，所述步骤S22中所述动物皮下注射进行免疫反应共进行4次；所述步骤S23中，经亲和层析处理后的多克隆抗体在透析袋中置于TBS缓冲液中，在4℃下透析过夜，所述TBS缓冲溶液包括含20mM的Tris、500mM的NaCl、0.05％的Tween-20，其pH为7.4。

10.根据权利要求9所述的用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其特征在于，所述步骤S32采用的缓冲溶液为50mM、pH 6.0的MES缓冲溶液或PBS缓冲溶液。

一种用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法

技术领域

[0001] 本发明属于医疗检测方法技术领域，涉及一种快速定量测定粪便中幽门螺杆菌抗原(尿素酶)的方法，具体地说涉及一种乳胶免疫比浊法快速定量测定粪便中幽门螺杆菌抗原的试剂的制备方法。

背景技术

[0002] 大量研究表明，幽门螺杆菌(Helicobacter pylori,Hp)感染是慢性胃炎、消化性溃疡的重要病因，且与胃癌、胃粘膜相关淋巴样组织淋巴瘤密切相关。目前，临床上已有多种方法评价Hp感染与否，主要分为两大类：侵入性和非侵入性检测方法。

[0003] 其中，侵入性检测方法是使用内窥镜取得胃活检组织后进行检测，一般包括脲酶试验法、病理学检测法及细菌培养法。快速脲酶试验法依据幽门螺杆菌具有丰富的可分解尿素的尿素酶，尿素酶可分解尿素产生氨气，通过 pH的变化测定幽门螺杆菌，该方法简便迅速，但是存在准确度不足的问题，可能因含尿素酶的其它细菌存在而产生假阳性或者检测近期试用过降低胃部细菌量或直接抑制尿素酶活性的药物的样品可产生假阴性。病理学检测法通过对胃粘膜组织切片染色检查，可直接显示幽门螺杆菌菌体而证实，但是不同病理学家观察的差异性对于胃萎缩样品的诊断具有一定的困难性。细菌培养法通过取胃粘膜组织做幽门螺杆菌培养，但是存在培养细菌较少、操作过程复杂、费用昂贵的问题。另外，侵入性检测方法还存在内窥镜侵入待检测者体内导致待检测者痛苦或易产生感染的问题。

[0004] 非侵入性检测方法指不通过胃镜取活检标本诊断幽门螺杆菌标本感染的方法。这类方法包括血清抗体检测、13C/14C呼气试验等。血清抗体检测目前已有的方法包括酶联免疫法、免疫印迹法、胶体金法等，但是由于根除幽门螺杆菌后人体血清中仍然含有对应的抗体，因此通过血清抗体来判定幽门螺杆菌感染存在特异性问题，不能真实反映出胃部是否还有幽门螺杆菌存在。13C/14C呼气试验作为一种检测幽门螺杆菌感染的方法，在临床上广泛应用，但是存在放射性问题，同时需要专门的设备，因此在家庭和社康中心的应用就受到很大限制。

[0005] 本发明采用粪便标本进行测试，通过专用的定量粪便取样器采集幽门螺杆菌抗原(尿素酶)，通过乳胶增强免疫比浊法测定幽门螺杆菌的含量，具有方便快捷并且清洁卫生的特点，可以在家庭和社康中心广泛应用。乳胶增强免疫比浊法是一种通过将抗体偶联到一定粒径的乳胶颗粒上，与标本中抗原反应后形成抗原-抗体-乳胶颗粒的复合物而形成一定的浊度，测定浊度的变化从而判定标本中抗原浓度的方法。目前，幽门螺杆菌已经被世界卫生组织确定为I类致癌病原，因此如何制备一种可快速无侵害性检测幽门螺杆菌的试剂成为目前亟待解决的问题。

发明内容

[0006] 为此，本发明所要解决的技术问题在于通过乳胶增强免疫比浊法，快速定量检测粪便中幽门螺杆菌抗原(尿素酶)。

[0007] 为解决上述技术问题，本发明的技术方案为：

[0008] 本发明提供一种用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，其包括制备R2试剂的步骤：

[0009] S1、提取幽门螺杆菌抗原蛋白，将尿素酶基因克隆到质粒中，通过大肠杆菌发酵表达，得到重组幽门螺杆菌抗原；

[0010] S2、制备抗幽门螺杆菌抗原多克隆抗体；

[0011] S3、制备包被抗幽门螺杆菌抗原多克隆抗体的乳胶颗粒；

[0012] S4、将包被抗幽门螺杆菌抗原多克隆抗体的乳胶颗粒与含有0.1％-10％电解质、0.1％-10％稳定剂、0.1％-10％表面活性剂、0.1％-5％防腐剂和1-1000mmol/L的MES缓冲液混合，即得。

[0013] 作为优选，所述步骤S1具体为，选择尿素酶做为幽门螺杆菌抗原，所述尿素酶包括UreA和UreB蛋白亚基，将UreA或UreB基因克隆到质粒中，通过大肠杆菌发酵表达以及亲和层析纯化，即得幽门螺杆菌抗原，所述质粒包括但不限于pGEX-4T-1质粒，所述质粒包含蛋白标签基因，所述蛋白标签包括但不限于GST蛋白。

[0014] 作为优选，所述步骤S2包括：

[0015] S21、将幽门螺杆菌抗原用等量弗氏完全佐剂乳化；

[0016] S22、将乳化后的抗原进行动物皮下注射，进行免疫反应；

[0017] S23、取经过免疫的血清进行亲和层析处理后即得抗幽门螺杆菌抗原多克隆抗体。

[0018] 作为优选，所述步骤S3包括如下步骤：

[0019] S31、将乳胶颗粒与缓冲溶液混合并加入表面活性剂，得到乳胶颗粒溶液，所述乳胶颗粒占所述乳胶颗粒溶液的质量百分比为0.01-10％；

[0020] S32、将所述抗幽门螺杆菌抗原多克隆抗体溶解于缓冲溶液得到抗体溶液，所述抗幽门螺杆菌抗原多克隆抗体占抗体溶液的质量百分比为0.01-10％；

[0021] S33、将所述乳胶颗粒溶液与所述抗体溶液混合后，加入1-(3-二甲氨基丙基)-3-乙基碳二亚胺盐酸盐，所述1-(3-二甲氨基丙基)-3-乙基碳二亚胺盐酸盐占混合溶液的质量百分比为0.01％-5％，室温下反应2-4小时，得到包被抗幽门螺杆菌抗原多克隆抗体的乳胶颗粒。

[0022] 作为优选，还包括制备R1试剂的步骤，所述R1试剂包括0.1％-10％的电解质、0.1％-10％的稳定剂、0.1％-10％的表面活性剂、0.1％-5％的防腐剂，余量为浓度1-1000mmol/L的MES缓冲液。

[0023] 作为优选，还包括制备校准品的步骤：以重组幽门螺杆菌抗原为母液，用牛血清蛋白溶液梯度稀释所述重组幽门螺杆菌抗原，即得不同浓度的校准品，所述牛血清蛋白溶液的浓度为1ng/ml-100g/l。

[0024] 作为优选，所述R1试剂、R2试剂中的所述电解质包括但不限于氯化钠，所述稳定剂包括但不限于甘露醇，所述表面活性剂包括但不限于吐温80，防腐剂包括但不限于叠氮钠。

[0025] 作为优选，所述乳胶颗粒的粒径为50-500nm。

[0026] 作为优选，所述步骤S22中所述动物皮下注射进行免疫反应共进行4次；所述步骤S23中，经亲和层析处理后的多克隆抗体在透析袋中置于TBS缓冲液中，在4℃下透析过夜，所述TBS缓冲溶液包括含20mM的Tris、500mM的 NaCl、0.05％的Tween-20，其pH为7.4。

[0027] 作为优选，所述步骤S32采用的缓冲溶液为50mM、pH 6.0的MES缓冲溶液或PBS缓冲溶液。

[0028] 本发明的上述技术方案相比现有技术具有以下优点：

[0029] 本发明所述的用于定量测定粪便中幽门螺杆菌抗原(尿素酶)试剂的制备方法，包括制备R2试剂的步骤：S1、提取幽门螺杆菌抗原(尿素酶)蛋白，将尿素酶基因克隆到pGEX-4T-1质粒中，通过大肠杆菌发酵表达，得到重组幽门螺杆菌抗原(尿素酶)；S2、制备抗幽门螺杆菌抗原(尿素酶)多克隆抗体；S3、制备包被抗幽门螺杆菌抗原(尿素酶)多克隆抗体的乳胶颗粒；S4、将包被抗幽门螺杆菌抗原(尿素酶)多克隆抗体的乳胶颗粒与含有0.1％-10％电解质、0.1％-10％稳定剂、0.1％-10％表面活性剂、0.1％-5％防腐剂和 1-1000mmol/L的MES缓冲液混合，即得到R2试剂。该方法制得的试剂是一种可直接通过粪便检测幽门螺杆菌的溶液，本发明采用粪便标本进行测试，通过乳胶增强免疫比浊法测定幽门螺杆菌的含量，具有方便快捷并且清洁卫生的特点，可以在家庭和社康中心广泛应用，仅需粪便样品即可完成检测，取样方便，对人体无安全隐患，检测效率高、稳定性好，特异性强，不易受干扰。

附图说明

[0030] 图1是本发明实施例中克隆位点的示意图；

[0031] 图2是本发明实施例中吸光度测试图；

[0032] 图3是本发明实施例中抗原标准品的标准曲线图。

具体实施方式

[0033] 为了使本发明的内容更容易被清楚的理解，下面根据本发明的具体实施例对本发明作进一步详细的说明。

[0034] 实施例1

[0035] 本实施提供一种用于定量测定粪便中幽门螺杆菌抗原的试剂的制备方法，所述试剂包括R1试剂和R2试剂，其中，以质量百分比计，所述R1试剂包含0.1-10％的电解质、0.1-10％的稳定剂、0.1-10％的表面活性剂、0.1-5％的防腐剂，余量为1-100mmol/L的MES缓冲液。

[0036] 1、制备R2试剂

[0037] 所述R2试剂包括包被抗幽门螺杆菌抗原(尿素酶)多克隆抗体的乳胶颗粒、电解质、稳定剂、表面活性剂、防腐剂和缓冲液，所述R2试剂通过如下工艺制备：

[0038] S1、选择尿素酶的任何一种蛋白亚基做为幽门螺杆菌抗原，尿素酶由两个蛋白亚基组成，分别是UreA和UreB，将UreA或UreB基因克隆到 pGEX-4T-1质粒中，克隆位点为pGEX-4T-1质粒中的BamHI和EcoRI(如图1所示)，所述pGEX-4T-1质粒含有GST(Glutathione-S-Transferase)蛋白标签基因，可以与尿素酶的任何一种蛋白亚基进行重组；所述UreA、UreB、 pGEX-4T-1质粒的基因序列见表1；将GST标签和尿素酶的UreA或UreB亚基蛋白进行连接，克隆尿素酶UreA或UreB基因的pGEX-4T-1质粒转化到大肠杆菌菌株中，通过大肠杆菌发酵表达出GST融合尿素酶(UreA蛋白或UreB蛋白)，然后用Glutathione Sepharose 4B凝胶(采购自GE公司) 进行亲和层析纯化，使用洗脱液体系为5mM谷胱甘肽

(GSH)的缓冲液 (含25mM Tris-HCl,100mM NaCl,1mM EDTA,1mM二硫苏糖醇,pH 7.4),然后把洗脱液放到透析袋中,再用透析液进行透析(25mM Tris-HCl, pH 7.4,100mM NaCl,1mM EDTA,1mM二硫苏糖醇),去除谷胱甘肽 (GSH),即得幽门螺杆菌抗原(GST融合尿素酶亚基蛋白),简称幽门螺杆菌抗原(尿素酶),最后幽门螺杆菌抗原(尿素酶)经过BCA法测定蛋白浓度,质量比为1.0mg/ml-10.0mg/ml。得到的幽门螺杆菌抗原(尿素酶) 可用于接下来的抗体制备,同时也可作为标准品和质控品的母液。

[0039] S2、制备幽门螺杆菌抗原(尿素酶)多克隆抗体:

[0040] S21、将100μg幽门螺杆菌抗原(尿素酶)以等量弗氏完全佐剂乳化。

[0041] S22、将其背部皮下多点注射于2.2～2.5kg的新西兰白兔体内,其后每隔两周以弗氏不完全佐剂乳化抗原,同法重复注射进行加强免疫3次,共计免疫4次。

[0042] S23、然后从静脉抽取100ml兔子血液,离心获得血清。取10ml兔血清通过免疫亲和层析柱,用1000ml TBS(含20mM Tris,500mM NaCl,0.05％ Tween-20,pH 7.4)缓冲液反复冲洗层析柱,弃去流出液,冲洗完毕后,用 10ml的glycine/HCl(100mM,pH 2.5)缓冲液洗脱结合的抗体,收集于透析袋中,并放置于1000ml TBS(含20mM Tris,500mM NaCl,0.05％ Tween-20, pH 7.4)缓冲液中,冰箱中4℃透析过夜,获得抗幽门螺杆菌抗原(尿素酶) 多克隆抗体。

[0043] S3、通过化学键联的方法制备包被抗幽门螺杆菌抗原(尿素酶)多克隆抗体的乳胶颗粒,具体包括:

[0044] S31、将100mg的乳胶颗粒与5ml MES缓冲液(50mM,pH 6.0)或PBS 缓冲液中混合后,加入表面活性剂十二烷基磺酸钠(最终浓度0.01％),得到乳胶颗粒溶液。

[0045] S32、将抗幽门螺杆菌抗原(尿素酶)多克隆抗体溶解于5ml MES缓冲液中(50mM,pH 6.0)或PBS缓冲液中,最终浓度达到1～10μmol/ml,得到抗幽门螺杆菌抗原(尿素酶)多克隆抗体溶液。

[0046] S33、将乳胶颗粒溶液和抗幽门螺杆菌抗原(尿素酶)多克隆抗体溶液充分混合,然后加入100mg的1-(3-二甲氨基丙基)-3-乙基碳二亚胺盐酸盐 (EDC)溶解于混合液中,室温下反应2～4小时,成功获得包被抗幽门螺杆菌抗原(尿素酶)多克隆抗体的乳胶颗粒。

[0047] S4、将4mg/mL的包被抗幽门螺杆菌抗原(尿素酶)多克隆抗体的乳胶颗粒与含有3％氯化钠、2.5％甘露醇、5％吐温80、0.5％叠氮钠和250mmol/L 的MES缓冲液(pH 7.4)混合,即得R2试剂。

[0048] 表1

[0049]

UreA	ATGAAACTCACCCCAAAAGAGTTAGACAAGTTGATGCTCCACTATGC TGGAGAATTGGCTAAAAAACGCAAAGAAAAAGGCATTAAGCTTAACT ATGTAGAAGCGGTAGCTTTGATTAGTGCCCATATTATGGAAGAAGCG AGAGCTGGTAAAAAGACTGCGGCTGAATTGATGCAAGAAGGGCGCAC TCTTTTAAAACCGGATGATGTGATGGATGGCGTGGCAAGCATGATCC ATGAAGTGGGTATTGAAGCGATGTTTCCTGATGGGACAAAACTCGTA ACCGTGCATACCCCTATTGAGGCCAATGGTAAATTAGTTCCTGGTGAG TTGTTCTTAAAAAATGAAGACATCACTATCAACGAAGGCAAAAAAGC CGTTAGCGTGAAAGTTAAAAATGTTGGCGACAGACCGGTTCAAATCG GCTCACACTTCCATTTCTTTGAAGTGAATAGATGCTTAGACTTTGACA GAGAAAAAACTTTCGGTAAACGCTTAGACATTGCGAGCGGGACAGCG GTAAGGTTTGAGCCTGGCGAAGAAAAATCCGTAGAATTGATTGACAT TGGCGGTAACAGAAGAATCTTTGGATTTAACGCGTTGGTTGATAGGC AAGCAGACAACGAAAGCAAAAAAATTGCTTTACACAGAGCTAAAGA GCGTGGTTTTCATGGCGCTAAAAGCGATGACAACTATGTAAAAACAA TTAAGGAGTAA
UreB	ATGAAAAAGATTAGCAGAAAAGAATATGTTTCTATGTATGGCCCTAC TACAGGCGATAAAGTGAGATTGGGCGATACAGACTTGATCGCTGAAG TAGAACATGACTACACCATTTATGGCGAAGAGCTTAAATTCGGTGGC GGTAAAACCCTGAGAGAAGGCATGAGCCAATCCAACAACCCTAGCAA AGAAGAATTGGATCTAATCATCACTAACGCTTTAATCGTGGATTACAC CGGTATTTATAAAGCGGATATTGGTATTAAAGATGGCAAAATCGCTG GCATTGGTAAAGGCGGTAACAAAGACATGCAAGATGGCGTTAAAAAC AATCTTAGCGTAGGTCCTGCTACTGAAGCCTTAGCCGGTGAAGGTTTG

[0050]

	ATCGTAACTGCTGGTGGTATTGACACACACATCCACTTCATTTCACCC CAACAAATCCCTACAGCTTTTGCAAGCGGTGTAACAACCATGATTGGT GGCGGAACTGGTCCTGCTGATGGCACTAATGCGACTACTATCACTCCA GGCAGAAGAAATTTAAAATGGATGCTCAGAGCGGCTGAAGAATATTC TATGAACTTAGGTTTCTTGGCTAAAGGTAACGCTTCTAACGACGCGAG CTTAGCCGATCAAATTGAAGCTGGTGCGATTGGCTTTAAAATCCACGA AGACTGGGGCACCACTCCTTCTGCAATCAATCATGCGTTAGATGTTGC AGACAAATACGATGTGCAAGTCGCTATCCACACAGACACTTTGAATG AAGCCGGTTGCGTGGAAGACACTATGGCAGCTATTGCCGGACGCACT ATGCACACTTTCCACACTGAAGGTGCTGGCGGCGGACACGCTCCTGAT ATTATTAAAGTAGCTGGTGAACACAACATTCTTCCCGCTTCCACTAAC CCCACTATCCCTTTCACTGTGAATACAGAAGCAGAACACATGGACAT GCTTATGGTGTGCCACCACTTGGATAAAAGCATTAAAGAAGATGTTC AGTTCGCTGATTCAAGGATCCGCCCTCAAACCATTGCGGCTGAAGAC ACTTTGCATGACATGGGGATTTTCTCAATCACCAGCTCTGACTCTCAA GCTATGGGTCGTGTGGGTGAAGTTATCACTAGAACTTGGCAAACAGC TGACAAAAACAAAAAAGAATTTGGCCGCTTGAAAGAAGAAAAAGGC GATAACGACAACTTCAGGATCAAACGCTACTTGTCTAAATACACCATT AACCCAGCGATCGCTCATGGGATTAGCGAGTATGTAGGTTCTGTAGA AGTGGGCAAAGTGGCTGACTTGGTATTGTGGAGTCCCGCATTCTTTGG CGTAAAACCCAACATGATCATCAAAGGCGGGTTCATTGCGTTGAGTC AAATGGGTGACGCGAACGCTTCTATCCCTACCCCACAACCAGTTTATT ACAGAGAAATGTTCGCTCATCATGGTAAAGCCAAATACGATGCAAAC ATCACTTTTGTGTCTCAAGCGGCTTATGACAAAGGCATTAAAGAAGA ATTAGGGCTTGAAAGACAAGTGTTGCCGGTAAAAAATTGCAGAAACA TCACTAAAAAAGACATGCAATTCAACGACACTACCGCTCACATTGAA GTCAATCCTGAAACTTACCATGTGTTCGTGGATGGCAAAGAAGTAACT TCTAAACCAGCCAATAAAGTGAGCTTGGCGCAACTCTTTAGCATTTTC TAG
pGEX-4 T-1 质粒	ACGTTATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC GGAAGCTGTG GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC GCACTCCCGT TCTGGATAAT GTTTTTTGCG CCGACATCAT AACGGTTCTG GCAAATATTC TGAAATGAGC TGTTGACAAT TAATCATCGG

[0051]

CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAATTTCA
CACAGGAAAC AGTATTCATG TCCCCTATAC TAGGTTATTG
GAAAATTAAG GGCCTTGTGC AACCCACTCG ACTTCTTTTG
GAATATCTTG AAGAAAAATA TGAAGAGCAT TTGTATGAGC
GCGATGAAGG TGATAAATGG CGAAACAAAA AGTTTGAATT
GGGTTTGGAG TTTCCCAATC TTCCTTATTA TATTGATGGT
GATGTTAAAT TAACACAGTC TATGGCCATC ATACGTTATA
TAGCTGACAA GCACAACATG TTGGGTGGTT GTCCAAAAGA
GCGTGCAGAG ATTTCAATGC TTGAAGGAGC GGTTTTGGAT
ATTAGATACG GTGTTTCGAG AATTGCATAT AGTAAAGACT
TTGAAACTCT CAAAGTTGAT TTTCTTAGCA AGCTACCTGA
AATGCTGAAA ATGTTCGAAG ATCGTTTATG TCATAAAACA
TATTTAAATG GTGATCATGT AACCCATCCT GACTTCATGT
TGTATGACGC TCTTGATGTT GTTTTATACA TGGACCCAAT
GTGCCTGGAT GCGTTCCCAA AATTAGTTTG TTTTAAAAAA
CGTATTGAAG CTATCCCACA AATTGATAAG TACTTGAAAT
CCAGCAAGTA TATAGCATGG CCTTTGCAGG GCTGGCAAGC
CACGTTTGGT GGTGGCGACC ATCCTCCAAA ATCGGATCTG
GTTCCGCGTG GATCCCCGGA ATTCCCGGGT CGACTCGAGC
GGCCGCATCG TGACTGACTG ACGATCTGCC TCGCGCGTTT
CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA
GACAAGCCCG TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG
TCGGGGCGCA GCCATGACCC AGTCACGTAG CGATAGCGGA
GTGTATAATT CTTGAAGACG AAAGGGCCTC GTGATACGCC
TATTTTTATA GGTTAATGTC ATGATAATAA TGGTTTCTTA
GACGTCAGGT GGCACTTTTC GGGGAAATGT GCGCGGAACC
CCTATTTGTT TATTTTTCTA AATACATTCA AATATGTATC
CGCTCATGAG ACAATAACCC TGATAAATGC TTCAATAATA
TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC
GCCCTTATTC CCTTTTTTGC GGCATTTTGC CTTCCTGTTT
TTGCTCACCC AGAAACGCTG GTGAAAGTAA AAGATGCTGA
AGATCAGTTG GGTGCACGAG TGGGTTACAT CGAACTGGAT
CTCAACAGCG GTAAGATCCT TGAGAGTTTT CGCCCCGAAG

[0052]

	AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG TGGCGCGGTA TTATCCCGTG TTGACGCCGG GCAAGAGCAA CTCGGTCGCC GCATACACTA TTCTCAGAAT GACTTGGTTG AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACTT ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCGCT TTTTTGCACA ACATGGGGGA TCATGTAACT CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA GCGTGACACC ACGATGCCTG CAGCAATGGC AACAACGTTG CGCAAACTAT TAACTGGCGA ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC AGGACCACTT CTGCGCTCGG CCCTTCCGGC TGGCTGGTTT ATTGCTGATA AATCTGGAGC CGGTGAGCGT GGGTCTCGCG GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCCTCCCG TATCGTAGTT ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCACT GAGCGTCAGA CCCCGTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG CTTGCAAACA AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACCTCGC TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT GGACTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCGTG CACACAGCCC AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG GGTCGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG

[0053]

TCCTGTCGGG TTTCGCCACC TCTGACTTGA GCGTCGATTT
TTGTGATGCT CGTCAGGGGG GCGGAGCCTA TGGAAAAACG
CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CCTTTTGCTG
GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT
TCTGTGGATA ACCGTATTAC CGCCTTTGAG TGAGCTGATA
CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT
GAGCGAGGAA GCGGAAGAGC GCCTGATGCG GTATTTTCTC
CTTACGCATC TGTGCGGTAT TTCACACCGC ATAAATTCCG
ACACCATCGA ATGGTGCAAA ACCTTTCGCG GTATGGCATG
ATAGCGCCCG GAAGAGAGTC AATTCAGGGT GGTGAATGTG
AAACCAGTAA CGTTATACGA TGTCGCAGAG TATGCCGGTG
TCTCTTATCA GACCGTTTCC CGCGTGGTGA ACCAGGCCAG
CCACGTTTCT GCGAAAACGC GGGAAAAAGT GGAAGCGGCG
ATGGCGGAGC TGAATTACAT TCCCAACCGC GTGGCACAAC
AACTGGCGGG CAAACAGTCG TTGCTGATTG GCGTTGCCAC
CTCCAGTCTG GCCCTGCACG CGCCGTCGCA AATTGTCGCG
GCGATTAAAT CTCGCGCCGA TCAACTGGGT GCCAGCGTGG
TGGTGTCGAT GGTAGAACGA AGCGGCGTCG AAGCCTGTAA
AGCGGCGGTG CACAATCTTC TCGCGCAACG CGTCAGTGGG
CTGATCATTA ACTATCCGCT GGATGACCAG GATGCCATTG
CTGTGGAAGC TGCCTGCACT AATGTTCCGG CGTTATTTCT
TGATGTCTCT GACCAGACAC CCATCAACAG TATTATTTTC
TCCCATGAAG ACGGTACGCG ACTGGGCGTG GAGCATCTGG
TCGCATTGGG TCACCAGCAA ATCGCGCTGT TAGCGGGCCC
ATTAAGTTCT GTCTCGGCGC GTCTGCGTCT GGCTGGCTGG
CATAAATATC TCACTCGCAA TCAAATTCAG CCGATAGCGG
AACGGGAAGG CGACTGGAGT GCCATGTCCG GTTTTCAACA
AACCATGCAA ATGCTGAATG AGGGCATCGT TCCCACTGCG
ATGCTGGTTG CCAACGATCA GATGGCGCTG GGCGCAATGC
GCGCCATTAC CGAGTCCGGG CTGCGCGTTG GTGCGGATAT
CTCGGTAGTG GGATACGACG ATACCGAAGA CAGCTCATGT
TATATCCCGC CGTTAACCAC CATCAAACAG GATTTTCGCC
TGCTGGGGCA AACCAGCGTG GACCGCTTGC TGCAACTCTC
TCAGGGCCAG GCGGTGAAGG GCAATCAGCT GTTGCCCGTC

[0054]

	TCACTGGTGA AAAGAAAAAC CACCCTGGCG CCCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT CATTAATGCA GCTGGCACGA CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA GTTAGCTCAC TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CTATGACCAT GATTACGGAT TCACTGGCCG TCGTTTTACA ACGTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG CACATCCCCC TTTCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCC AACAGTTGCG CAGCCTGAAT GGCGAATGGC GCTTTGCCTG GTTTCCGGCA CCAGAAGCGG TGCCGGAAAG CTGGCTGGAG TGCGATCTTC CTGAGGCCGA TACTGTCGTC GTCCCCTCAA ACTGGCAGAT GCACGGTTAC GATGCGCCCA TCTACACCAA CGTAACCTAT CCCATTACGG TCAATCCGCC GTTTGTTCCC ACGGAGAATC CGACGGGTTG TTACTCGCTC ACATTTAATG TTGATGAAAG CTGGCTACAG GAAGGCCAGA CGCGAATTAT TTTTGATGGC GTTGGAATT

[0055] 2、制备R1试剂

[0056] 所述R1试剂由质量浓度为3％的氯化钠、质量浓度为2.5％的甘露醇、质量浓度为5％的吐温80、质量浓度为0.5％的叠氮钠和摩尔浓度为250mmol/L的MES缓冲溶液(pH 7.4)组成，按比例将上述组分混合均匀即得。

[0057] R1、R2试剂制备后，分别置于R1试剂盒和R2试剂盒中。

[0058] 3、制备校准品

[0059] 以通过步骤S1所述的基因重组技术提纯的幽门螺杆菌抗原(尿素酶) 为母液，使用牛血清蛋白梯度稀释母液，制备不同浓度的校准品：S5:40 ng/mL，S4:20ng/mL，S3:10ng/mL，S2:5ng/mL，S1:2.5ng/mL，其中牛血清白蛋白浓度为1ng/ml-100g/l。

[0060] 4、制备质控品

[0061] 以通过步骤S1中所述的基因重组技术提纯的幽门螺杆菌抗原(尿素酶) 为母液，使用牛血清白蛋白梯度稀释母液，制备两种浓度的质控品：C1： 4.0ng/mL，C2:1.0ng/mL，其中牛血清白蛋白浓度1ng/ml-100g/l。

[0062] 5、标准曲线的制定

[0063] 试剂盒测定使用全自动生化分析仪检测主波长：450nm，副波长： 800nm。

[0064] 试剂用量：粪便样本15μl；R1试剂305μl；R2试剂25μl，所述粪便样本通过含有如下成分的裂解液处理后得到：20mM Tris-HCl(pH7.5)，1 mM DTT，2mM EDTA，2mM EGTA，25mM NaF，25mMβ-glycerophosphate， 0.1mM Na3VO4，0.5mM苯甲基磺酰氟(PMSF)和0.3％ Nonidet P-40。

[0065] 测定方法(两点终点法)：305μl R1试剂加入15μl样本，于37℃反应5分钟，然后加

入25μl R2试剂即开始读点测得吸光度A1，10分钟之后再次读点测得吸光度A2，计算吸光度差值ΔA＝A2-A1，如图2所示。

[0066] 制作标准曲线：采用制得的幽门螺杆菌抗原(尿素酶)校准品，浓度分别为S5：40ng/mL，S4：20ng/mL，S3：10ng/mL，S2：5ng/mL，S1：2.5 ng/mL。按照上述步骤测得本发明幽门螺杆菌抗原(尿素酶)标准品的标准曲线，如图3所示。图3中曲线上的每个点代表一个含量的标准品，其中x 轴表示幽门螺杆菌抗原(尿素酶)的浓度，y轴表示吸光度的差值。

[0067] 6、线性范围的确定

[0068] 将接近线性范围上限的幽门螺杆菌抗原(尿素酶)高浓度样本100 ng/mL，用生理盐水按1/2、1/4、1/8、1/16、1/32、1/64稀释，共配制成7 个不同浓度的溶液，另以不含幽门螺杆菌抗原(尿素酶)的生理盐水作为空白溶液。用制作标准曲线的方法检测各浓度，将测定浓度值与理论浓度进行线性回归分析，计算回归方程为：y＝1.0214x+2.2577，相关系数r＝0.986，表明本发明试剂盒在0～100ng/mL线性范围内相关性较好。

[0069] 7、准确度测定

[0070] 采用全自动生化分析仪对20例人进行粪便测定，样本来自深圳市大鹏新区葵涌人民医院。参考值：小于等于0.5ng/mL为阴性标本，大于1.0ng/mL 为阳性标本，介于0.5-1.0ng/mL数值为不确定标本，需要重新测试，测试结果如表2所示。

[0071] 表2

[0072]

序号	本发明试剂盒测定粪便值（ng/mL）	临床病症表现（C13 吹气）	序号	本发明试剂盒测定粪便值（ng/mL）	临床病症表现（C13 吹气）
1	29.1	阳性	11	0.1	阴性
2	0.3	阴性	12	3.9	阳性
3	0.11	阴性	13	0.4	阴性
4	0.2	阴性	14	6.3	阳性
5	3.2	阳性	15	0.2	阴性
6	9.1	阳性	16	0.1	阴性
7	0.1	阴性	17	7.2	阳性
8	1.7	阳性	18	1.1	阳性
9	0.3	阴性	19	0.2	阴性
10	0.2	阴性	20	1.8	阳性

[0073] 上述结果表明，所制得的试剂与临床病症的相关性高。

[0074] 8、灵敏度测定

[0075] 灵敏度定义为单位浓度吸光度的变化，用幽门螺杆菌抗原(尿素酶)校准品对幽门螺杆菌抗原(尿素酶)试剂在全自动生化分析仪上定标，记录校准品(浓度为10ng/mL)与试剂反应的吸光度值为0.08，即该试剂在校准浓度为10ng/mL时的灵敏度为0.08，灵敏度高。

[0076] 9、批内精密度的测定

[0077] 按本发明所述的试剂测定同一份样本10次，计算测定均值和批内精密度，测试结果如表3所示。

[0078] 表3

[0079]

序号	浓度（ng/mL）	序号	浓度（ng/mL）
1	8.5	6	8.7
2	8.8	7	8.6
3	8.5	8	8.4
4	8.6	9	8.8

[0080]

5	8.9	10	8.5
均值（ $\bar{X}$ ）	8.63		
标准偏差（SD）	0.163639		
批内精密度（CV）	1.896%		

[0081] 10、抗干扰分析

[0082] 干扰物选择配方和测试结果如表4所示。

[0083] 表4

[0084]

加入的的干扰物质	测定值（ng/mL）	试验组与对照组测定值偏差
对照组：样本+蒸馏水	6.3	-
试验组：样本+VC	6.3	0%
试验组：样本+胆红素	6.2	1.59%
试验组：样本+血红蛋白	6.5	3.17%
试验组：样本+甘油三酯	6.2	1.59%

[0085] 上述结果表明本发明所述的方法制得的试剂抗干扰能力良好。

[0086] 显然，上述实施例仅仅是为清楚地说明所作的举例，而并非对实施方式的限定。对于所属领域的普通技术人员来说，在上述说明的基础上还可以做出其它不同形式的变化或变动。这里无需也无法对所有的实施方式予以穷举。而由此所引伸出的显而易见的变化或变动仍处于本发明创造的保护范围之中。

序列表

<110> 深圳市鸿美诊断技术有限公司

<120> 一种用于定量测定粪便中幽门螺杆菌抗原的试剂的制备方法

<130> JY181-277306

<141> 2018-09-30

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2325 2330 2335
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2340 2345 2350
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2355 2360 2365
Cys Thr Gly Ala Gly Cys Gly Thr Cys Ala Gly Ala Cys Cys Cys Cys

2370 2375 2380

Gly Thr Ala Gly Ala Ala Ala Ala Gly Ala Thr Cys Ala Ala Ala Gly
2385 2390 2395 2400

Gly Ala Thr Cys Thr Thr Cys Thr Thr Gly Ala Gly Ala Thr Cys Cys
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Thr Thr Thr Thr Thr Thr Thr Cys Thr Gly Cys Gly Cys Gly Thr Ala
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Ala Thr Cys Thr Gly Cys Thr Gly Cys Thr Thr Gly Cys Ala Ala Ala
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Cys Ala Ala Ala Ala Ala Ala Ala Cys Cys Ala Cys Cys Gly Cys Thr
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Cys Gly Ala Thr Ala Ala Gly Thr Cys Gly Thr Gly Thr Cys Thr Thr
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Gly Ala Cys Gly Ala Thr Ala Gly Thr Thr Ala Cys Cys Gly Gly Ala
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Cys Cys Cys Cys Thr Gly Ala Thr Thr Cys Thr Gly Thr Gly Gly Ala

3075 3080 3085

Thr Ala Ala Cys Cys Gly Thr Ala Thr Thr Ala Cys Cys Gly Cys Cys

3090 3095 3100

Thr Thr Thr Gly Ala Gly Thr Gly Ala Gly Cys Thr Gly Ala Thr Ala

3105 3110 3115 3120

Cys Cys Gly Cys Thr Cys Gly Cys Cys Gly Cys Ala Gly Cys Cys Gly

3125 3130 3135

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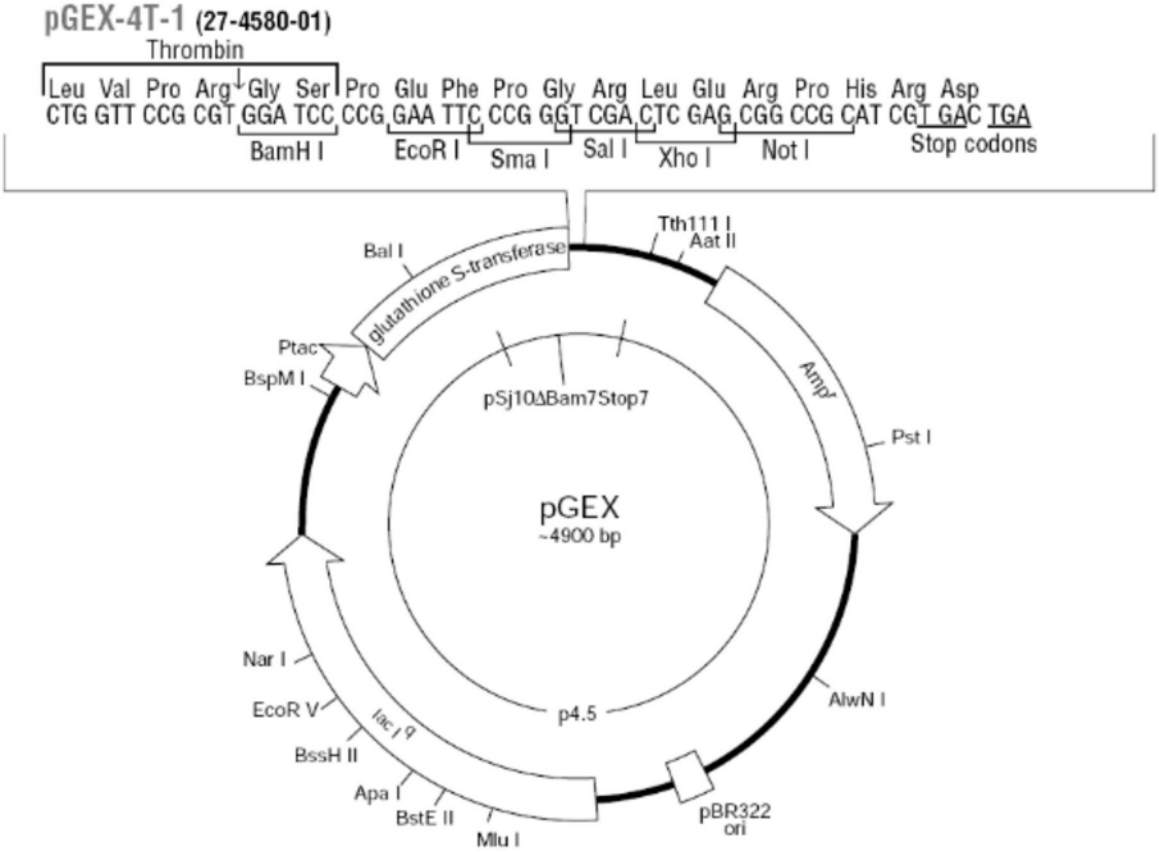
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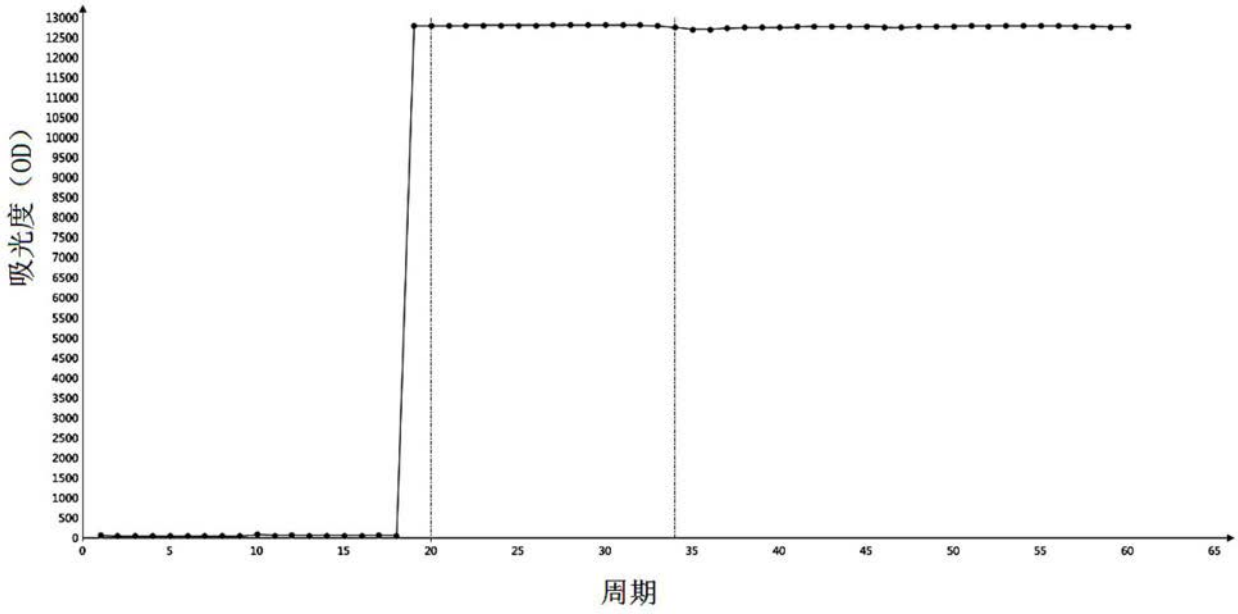
BamH I
EcoR I
Sma I
Sal I
Xho I
Not I
Stop codons
Bal I
glutathione S-transferase
Tth111 I
Aat II
Ptac
BspM I
pSj10ΔBam7Stop7
Amp
Pst I
pGEX
~4900 bp
Nar I
EcoR V
lac I q
BssH II
Apa I
BstE II
Mlu I
p4.5
AlwN I
pBR322
ori

图1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1500
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2500
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4500
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7000
7500
8000
8500
9000
9500
10000
10500
11000
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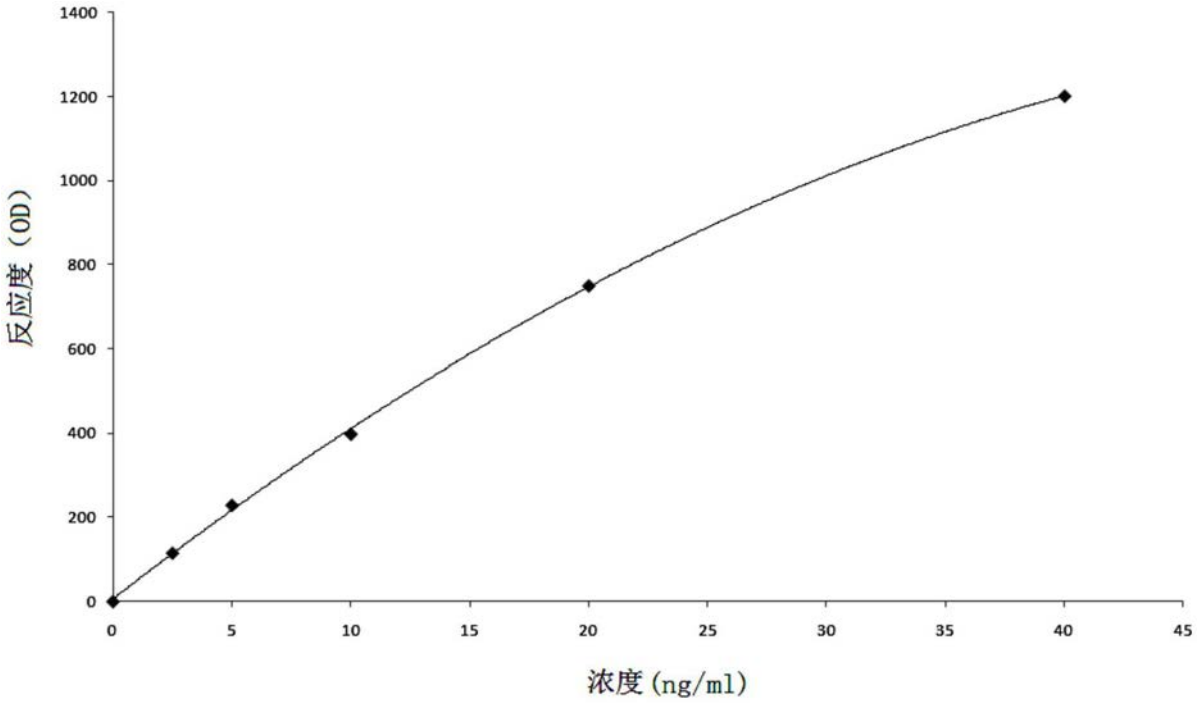
图2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1200
1000
800
600
400
200
0
反应度（OD）
0
5
10
15
20
25
30
35
40
45
浓度(ng/ml)

图3

专利名称(译)	一种用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法		
公开(公告)号	CN109342722A	公开(公告)日	2019-02-15
申请号	CN201811158165.6	申请日	2018-09-30
[标]申请(专利权)人(译)	深圳市鸿美诊断技术有限公司		
申请(专利权)人(译)	深圳市鸿美诊断技术有限公司		
当前申请(专利权)人(译)	深圳市鸿美诊断技术有限公司		
[标]发明人	侯志波 张伟 温建超		
发明人	侯志波 张伟 温建超 翁琪墁		
IPC分类号	G01N33/569 G01N33/531		
CPC分类号	G01N33/56911 G01N33/531		
代理人(译)	龙丹丹		
外部链接	Espacenet SIPO		

摘要(译)

本发明公开了一种用于定量测定粪便中幽门螺杆菌抗原试剂的制备方法，包括制备R2试剂的步骤：S1、提取幽门螺杆菌抗原(尿素酶)蛋白，将尿素酶基因克隆到pGEX-4T-1质粒中，通过大肠杆菌发酵表达，经过亲和层析纯化得到重组幽门螺杆菌抗原(尿素酶)；S2、制备抗幽门螺杆菌抗原(尿素酶)多克隆抗体；S3、制备包被抗幽门螺杆菌抗原(尿素酶)多克隆抗体的乳胶颗粒；S4、分散于缓冲溶液得到R2试剂。该方法是乳胶增强免疫比浊法，制得的试剂是一种可直接通过粪便检测幽门螺杆菌的溶液，仅需粪便样品即可完成检测，取样方便，对人体无安全隐患，检测效率高、稳定性好，特异性强，不易受干扰。