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IMPROVE 陽普醫療 IMPROVE MEDICAL

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文献导读

本期的文献导读是一篇专家综述《静脉血和末梢血在临床检测项目中的差别应用》。随着临床检测向着现代化、数字化和智能化的方向发展，检验医学逐步拓展了临检样本的多样性。但是在变化中，常规检测项目所常用的样本中，来自静脉的血液样本仍然是临床检测样本中的金标准。来自手指、耳垂等末端毛细血管的末梢血液样本是静脉血的方便简易的替代品。静脉血检测的结果准确、重复性好，是多项临检指南的基础。如果静脉血的结果和末梢血的结果相左，一般来说应该以静脉血结果为准。但是在另一方面，静脉血的采集的创伤性强，需要专业人员操作，在方便实用性上不如末梢血。究竟哪些临检项目可以采用末梢血，哪些项目必须采用静脉血，是临检领域的常见话题。

静脉血和末梢血在临床检测项目中的差别应用

董彩霞，李小林
GIMDx Inc.

在临床检验中，检测样本的选择通常对检测结果有着重要的影响。常用的检测样本包括全血、血浆、血清、尿液、唾液、脱落细胞和组织等，而这其中来自静脉的血样样本（简称“静脉血”），是临床检验样本中的最常用的样本。来自手指、耳垂或脚底等末端毛细血管的血样样本（简称“末梢血”），因为其容易采集，成本较低等特点，有时可作为静脉血的替代品。然而究竟哪些临床检验可以采用末梢血，哪些必须采用静脉血，仍是临床领域的持久研究话题。而在本期文章中，我们将主要根据近期的英文文献中的进展，介绍静脉血和末梢血对多种疾病诊断的影响。

静脉血和末梢血对病毒的检测

麻疹（Measles）是一种由病毒引起的传染性极强的严重疾病，而对麻疹的预防和治疗也一直是临床领域的重要课题之一[1]。早在 2003 年，美国 Mayo 疫苗研究小组（Mayo Vaccine Research Group），就针对是否可以使用末梢血去代替静脉血检测抗体的水平进行研究[1]。其研究结果表明通过对其中麻疹酶免疫测定，其中 85%是免疫的，而通过最小二乘线性回归法得到的末梢血和静脉血的相关系数是 0.98，而且通过这两种样本检测显示的呈免疫阳性个体的数量也是相同的[2]，因此可得出结论：对麻疹而言，静脉血和末梢血检测的抗体水平是没有显著差别的。

寨卡（Zika）病毒是一种蚊虫黄病毒，在 1947 年首次从乌干达的寨卡森林中的恒河猴中分离出来，后来在非洲森林的蚊子中发现并大量转播，是流行和突发公共卫生事件的焦点[3,4]。寨卡病毒是黄病毒科中一种阳性感应单链 RNA 病毒[5]，而单独的临床评估检测对于寨卡病毒感染的诊断是不可靠的，由于与其他虫媒病毒的临床重叠，目前两种广泛商业应用的化验方法是由欧共体批准的基于 PCR 的测定和美国食品药品监督管理局（FDA）批准的血清学测定法[6,7]。在 2017 年，美国一研究小组对比了静脉血和末梢血中携带的寨卡病毒的含量，研究从 21 位携带寨卡病毒的患者中进行了静脉血和末梢血血清取样，结果显示末梢血血清中携带的寨卡病毒含量高于静脉血[8]，也就是说末梢血样本对于寨卡病毒的检出，其灵敏度等于或者高于静脉血样。

败血症患者动脉血和静脉血中乳酸含量

败血症是一种常见的急诊症状，而对患者乳酸含量的监控对这类病症是很重要的，目前临床上的金标准是检测动脉血样本中乳酸（A-LACT）的含量，但由于动脉血采样过程比较繁琐，研究人员尝试用其他血液样本来替代[9,10]。虽然目前检测外周静脉血样本中乳酸（PV-LACT）含量的频率在增加，但是只有少数研究证明 A-LACT 和 PV-LACT 含量是可以相互替代的。如果能证明 PV-LACT 和 A-LACT 是相互可替换的关

系，那就能减轻患者在取样时的不舒适感、加快检测速度以及减少所需要的费用成本[11,12,13]。

基于以上原因，英国一个科研小组对 PV-LACT 和 A-LACT 含量关系进行了研究，研究通过对 304 位患者进行采样检测，研究结果发现乳酸含量在两种样本中的平均差别只有 0.4 mmol/L[14]。而与此同时苏格兰的研究小组也对这个课题进行了研究，他们采用了相对较小的样本数量，对 37 位患者进行了样本采集，结果显示乳酸含量在这两种样本中的平均差别只有 0.54 mmol/L[15]。以上这两组数值相对较小，这表示 PV-LACT 和 A-LACT 含量在某种角度来讲是可以相互替代的。

静脉血和末梢血取样中生育激素的含量

现如今微创指尖采样技术允许在家里检测生育激素水平，美国一研究小组检测并比较了静脉血和末梢血样本中激素的含量差别，研究对 130 名 18 ~ 40 岁之间的女性进行静脉血和末梢血样本的采集，研究结果发现静脉血和末梢血样本中激素的含量在所有测定范围内均有一致性而且呈现线性关系。静脉血和末梢血样本中检测的每一种激素的相关性达到 0.99 ~ 1.0，每一种化验结果都呈现高度的精确性（变异系数 < 13%）和高度的准确性（平均恢复值高达 95.5% ~ 102.3%）。基于此项研究结果，可得知静脉血和末梢血样本可互相替换用来检测抗苗勒氏管激素、E2、FSH、LH、PRL、睾酮、TSH 和游离 T4 含量[16,17]。

静脉血和末梢血中丙氨酸氨基转移酶（ALT）的快速测量

在 2015 年，美国 Beth Israel Deaconess 医疗中心就最近开发的一种基于试纸多重微流体性能检测静脉血血清和末梢血中丙氨酸氨基转移酶（ALT）含量的方法进行了检测与评估[18,20]。在这项研究中，对 96 名拥有不同丙氨酸氨基转移酶（ALT）浓度基线的门诊患者进行了末梢血样本采集，对其中 93 名患者进行了静脉血血清采集。研究数据显示通过这种试纸检测方式检测到在末梢血中 ALT 的含量低于对应静脉血血清中 ALT 的含量，而通过这项研究得到的结论是这种试纸检测对静脉血血清中 ALT 含量的检测是高度准确的，而对于末梢血中 ALT 含量的检测这种试纸检测存在系统性差异[21]。

抗阻力运动后静脉血和末梢血中总肌酸激酶含量

通常情况下，不正确的抗阻力运动可能会导致骨骼肌损伤，从而使总肌酸激酶活动增加[22,23]。美国一项研究评估了是否可以采用末梢血样本替代静脉血样本检测抗阻力运动后总肌酸激酶含量，其中 15 名未经训练的志愿者进行了 50 次最大偏心肘伸展，分别采集运动后 0 小时、24 小时、48 小时、72 小时和 96 小时的末梢血和静脉血样本。研究结果显示在以上几个时间段中静脉血和末梢血样本中总肌酸激酶含量的差异 < 12%，由于两种样本间的差异很小，美国研究人员表示科研人员及医疗人员可考虑使用末梢血替代静脉血去测量在抗阻力运动后总肌酸激酶的含量[24,25]。

静脉血和末梢血中乳酸含量

血液中的乳酸含量是用来检测患者疾病严重程度的标志，乳酸含量可以通过对以下三种血液样本进行采样检测：静脉血、末梢血和动脉血，现已证明静脉血和动脉血中的乳酸含量是可以相互替换的关系[14,26,27,28]，然而虽然对末梢血中乳酸（CAP-LACT）含量的测量可以改善患者疾病筛查，但由于其临床效用证据不足，还未能应用。因此英国研究人员对末梢血中乳酸（CAP-LACT）和外周静脉血中乳酸（PV-LACT）的关系进行了试验，试验抽取了 99 位病人的末梢血和静脉血样本进行 CAP-LACT 和 PV-LACT 含量检测，但结果发现 CAP-LACT 和 PV-LACT 的含量并不相同，所以就现在而言，临床医疗并不能用 CAP-LACT 去替代 PV-LACT 对患者进行乳酸含量的检测[29]。

末梢血和静脉血液样本测定心肌肌钙蛋白

心肌肌钙蛋白（cardiac troponin）是一种具有特异性和敏感性的心肌损伤生物标志物，是评估疑似急性心肌梗死的首选血清学检查，目前此项检测是采用静脉血样本进行检测的[30]。在 2013 年，美国一科研小组尝试用末梢血代替静脉血检测心肌肌钙蛋白含量，研究对 89 位患者进行了静脉血和末梢血样本采集和检测，其中 4 个样本出现了墨盒错误，剩余的 85 个样本中，通过对比末梢血和静脉血结果，显示有一个阳性预测值为 1.00，一个阴性预测值为 0.96，其敏感度为 0.625，且两种样本结果呈线性相关[31]。通过以上数据可得出的结论是末梢血还并不准确，所以不能用来检测心肌肌钙蛋白含量。

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Article Abstract Collection

文献摘要

本期的文献摘要，选取了专家综述中的若干重要的引用文献，针对其摘要做了中文翻译。这些文献代表了国际上对静脉血和末梢血检测的比较学研究，具体包括的领域是：静脉血和末梢血对部分种类病毒的检测，败血症患者动脉血和静脉血中乳酸含量，静脉血和末梢血取样中生育激素的含量，静脉血和末梢血中丙氨酸氨基转移酶的快速测量，离心运动后静脉血和末梢血中总肌酸激酶含量，静脉血和末梢血中乳酸含量，以及末梢血和静脉血液样本对心肌肌钙蛋白测定。

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摘要

我们比较了使用血清和滤纸血斑作为标本来源检测麻疹和风疹特异性 IgM 和 IgG。我们将 60 名儿童和 60 名健康成人的末梢血收集到微量管中和滤纸斑点上。在初次接种麻疹、腮腺炎、风疹疫苗后约 3 周，采集 12 ~ 15 个月大的儿童血液，并通过使用捕获抗体 EIA 和间接 EIA 测试样本对的麻疹特异性 IgM 和 IgG 抗体。我们通过使用市售的捕获 IgM（Captia）和间接 IgG（Wampole）试验来测试来自参与者子集的样品对的风疹特异性 IgM 和 IgG 抗体。来自血清和滤纸血斑的所有试验的结果均显示出高度一致性：麻疹 IgM 为 98%，麻疹 IgG 为 93%，风疹 IgM 为 94%，风疹 IgG 为 93%，排除不确定的样品后，四种试验的一致性均增加至 96% ~ 100%。麻疹 IgM 和 IgG 的 EIA 信号相关系数分别为 0.99 和 0.77，风疹 IgM 和 IgG 分别为 0.92 和 0.94。除了风疹 IgM 试验之外，其他测试用于滤纸样品的截止值与用于血清样品的截止值相同。滤纸血斑的使用是检测麻疹和风疹特异性抗体的一种很有前景的选择。

2. **NA, TR, RM, RA, GA, P., Loepfe, Jacobson, Vierkant, Poland (2003). Comparison of fingerstick versus venipuncture for antibody testing of measles and rubella. Scand J Infect Dis 35, 107-109.**

摘要

这项研究的目的是确定手指针刺获取血液用于测量抗体水平是否是静脉穿刺可接受的替代方法。通过静脉穿刺从健康成人受试者获得用于抗体测定的血液样本，通过标准方法从血块中分离血清。还通过刺穿第二或第三手指并通过微量血清分离管收集以获得手指针刺样品。然后通过离心分离血清，并立即将所有样品储存在-80°C直至测定。对于麻疹和风疹抗体检测，从手指针刺和静脉穿刺收集的血清之间存在极好的相关性。对于麻疹酶免疫测定（EIA），85%的受试者是免疫的，使用最小二乘线性回归，手指针刺和静脉穿刺结果之间的相关系数为 0.98。对于风疹 EIA，100%的受试者具有免疫力，相关系数为 0.83。两种方法均显示相同数量的免疫阳性个体；此外，没有仅在 1 种标本来源中检测到对两种病毒都没有免疫力的病例。通过两种方法获得的血液的抗体水平没有显著差异。

3. **Plourde, A.R., and Bloch, E.M. (2016). A literature review of zika virus. Emerging Infect. Dis. 22, 1185-1192.**

摘要

寨卡病毒是一种蚊虫传播的黄病毒，是持续大流行和突发公共卫生事件的焦点。以前仅在非洲和亚洲有散发病例，2015 年巴西寨卡病毒的出现预示着整个美洲的迅速传播。尽管大多数寨卡病毒感染的特征是亚临床或轻度流感样疾病，但也已有严重临床表现的描述，包括成人的格林-巴利综合征和感染母亲所生婴儿的小头畸形。寨卡病毒既没有有效的治疗方法，也没有疫苗可用；因此，公共卫生应对措施主要侧重于预防感染，尤其是在孕妇中。尽管对这种病毒的了解越来越多，但关于病毒的载体和宿主、发病机制、遗传多样性以及与其他循环病毒共感染的潜在协同效应仍存在疑问。这些问题突出了在目前的寨卡病毒流行病中优化监测、患者管理和公共卫生干预的研究需求。

4. **Lanciotti, R.S., Kosoy, O.L., Laven, J.J., Velez, J.O., Lambert, A.J., Johnson, A.J., Stanfield, S.M., and Duffy, M.R. (2008). Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerging Infect. Dis. 14, 1232-1239.**

摘要

寨卡病毒（ZIKV）是一种蚊媒黄病毒，于 1947 年在乌干达首次从哨兵猴身上分离出来。蚊子和哨兵动物监测研究表明 ZIKV 是非洲和东南亚的流行病，但报道的人类病例很罕见，文献报道 < 10 例。2007 年 6 月，在密克罗尼西亚联邦州的亚普州发现了与 ZIKV 有关的发烧和皮疹流行病。我们报告了与此流行病相关的 ZIKV 的遗传学和血清学特性。

5. **Hamel, R., Dejarnac, O., Wichit, S., Ekchariyawat, P., Neyret, A., Luplertlop, N., Perera-Lecoin, M., Surasombatpattana, P., Talignani, L., Thomas, F., et al. (2015). Biology of zika virus infection in human skin cells. J. Virol. 89, 8880-8896.**

摘要

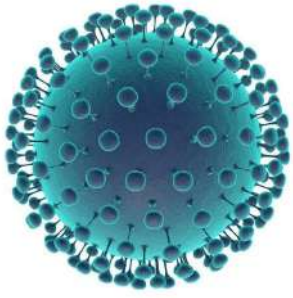
寨卡病毒（ZIKV）是黄病毒科的一种新兴虫媒病毒，黄病毒科包括登革热病毒、西尼罗病毒、黄热病毒和日本脑炎病毒，ZIKV 引起由伊蚊属传播的蚊媒疾病，最近在南太平洋爆发。在这里，我们研究了人类皮肤在 ZIKV 进入中的重要性及其对诱导抗病毒免疫反应的贡献。我们指出，人类皮肤成纤维细胞、表皮角质形成细胞和未成熟树突细胞是最近引起波利尼西亚流行病的 ZIKV 分离株几种进入因子和/或粘附因子，包括 DC-SIGN、AXL、Tyro3，以及 TIM-1（在较小程度上），允许 ZIKV 进入，主要对 TAM 受体 AXL 起作用。通过使用中和抗体和特异性 RNA 消噪，证实了人皮肤成纤维细胞对 ZIKV 的容纳性。ZIKV 诱导 Toll 样受体 3（TLR3）、RIG-I 和 MDA5，以及几种干扰素刺激的基因，包括 OAS2、ISG15 和 MX1 的转录，其特征在于强烈增强的 β 干扰素基因表达。发现 ZIKV 对 I 型和 II 型干扰素的抗病毒作用敏感。最后，皮肤成纤维细胞的感染导致自噬体的形成，其存在与增强的病毒复制相关，如使用 Torin 1（自噬的化学诱导剂）和特异性自噬抑制剂 3-甲基腺嘌呤所示。本文提供的结果使我们能够进一步了解 ZIKV 的生物学性质，并制定策略旨在干扰由这种新出现的黄病毒引起的病理。

重要性：寨卡病毒（ZIKV）是一种属于黄病毒科（Flaviviridae）的虫媒病毒。当食血的雌性伊蚊将病毒注射到其哺乳动物宿主的皮肤中，然后通过特定受体感染容纳细胞时，载体介导的 ZIKV 传播就开始了。实际上，皮肤免疫细胞，包括真皮成纤维细胞、表皮角质形成细胞和未成熟树突细胞，都被发现允许 ZIKV 感染。该结果还指出了磷脂酰丝氨酸受体 AXL 作为 ZIKV 进入受体的主要作用，以及容纳细胞中细胞自噬在增强 ZIKV 复制中的主要作用。ZIKV 复制导致抗病毒先天免疫应答的激活和感染细胞中 I 型干扰素的产生。总之，这些结果提供了对 ZIKV 与其哺乳动物宿主之间相互作用的首个一般性见解。

6. **Faye, O., Freire, C.C.M., Iamarino, A., Faye, O., de Oliveira, J.V.C., Diallo, M., Zanotto, P.M.A., and Sall, A.A. (2014). Molecular evolution of Zika virus during its emergence in the 20(th) century. PLoS Negl. Trop. Dis. 8, e2636.**

摘要

寨卡病毒（ZIKV）是一种蚊媒黄病毒，于 1947 年在乌干达首次分离。尽管昆虫学和病毒学监测报告了非洲和亚洲不同国家的 ZIKV 地方性活动，但直到 2007 年才报道了人类病例，当时在密克罗尼西亚发生了流行性寨卡热。在西非，世界卫生组织达喀尔巴斯德研究所的虫媒病毒和出血热合作中心（http：//www.pasteur.fr/recherche/banques/CRORA/）报告了自 1968 年以来 ZIKV 的周期性传播。尽管有几篇关于 ZIKV 的报道，但对来自西非的病毒株之间的遗传关系仍然知之甚少。为了评估病毒传播及其分子流行病学，我们调查了 1968 年 ~ 2002 年在塞内加尔和科特迪瓦六个地区收集的 37 株 ZIKV 分离株。此外，还包括了来自其他六个国家的毒株。我们的研究结果表明，西非这两个国家在 20 世纪至少经历过两次独立的 ZIKV 引入，显然这些病毒谱系不受蚊子载体种类的限制。此外，我们提出证据表明 ZIKV 可能在自然界中经历了重组，并且包膜蛋白中 N154 糖基化位点的丧失是对伊蚊（Aedes dalzieli）载体可能的适应性反应。



7. **Matheus, S., de Laval, F., Moua, D., N'Guyen, C., Martinez, E., Rousset, D., and Briolant, S. (2017). Zika virus persistence and higher viral loads in cutaneous capillaries than in venous blood. Emerging Infect. Dis. 23,.**

摘要

我们收集了在症状出现后的多天的 21 名寨卡病毒感染患者的静脉和末梢血清样本，发现末梢血中的 RNA 负荷比静脉血高，病毒检测的中位持续时间也显著长于静脉血。这些发现提出了关于末梢血在病毒传播动力学中的作用的问题。

8. **Polat, G., Ugan, R.A., Cadirci, E., and Halici, Z. (2017). Sepsis and septic shock: current treatment strategies and new approaches. Eurasian J. Med. 49, 53-58.**

摘要

脓毒症是一种复杂病症，其特征在于响应微生物损伤同时激活炎症和凝血。这些事件通过释放来自免疫细胞和/或受损内皮的促炎因子、促凝血剂和黏附因子而表现为全身性炎症反应综合征或败血症症状。现在，败血症是一种严重的多系统疾病，其临床表现难以治疗且死亡率高。特别是在过去的二十年中，已经对败血症进行了许多研究，败血症特别通过引起血液动力学变化造成休克、多器官功能障碍和器官衰竭。在败血症中，抗生素耐药性和耐药的血液动力学变化，推动了针对除经典治疗外的新治疗方式的进一步研究。在过去的十年中，脓毒症的病理生理学已被阐明。除了抗菌疗法之外，还使用了各种治疗剂，但没有获得令人满意的结果。本综述总结了败血症的病理生理学，以及现行治疗方案和新方法。

9. **C, R. (1991). The Pathogenesis of Sepsis. Ann Intern Med 115, 457-469.**

摘要

脓毒症及其后遗症（败血症综合征和脓毒性休克）越来越常见，并且仍然是可能致命的诊断。最近已经描述了脓毒症发病机理的许多介质，包括肿瘤坏死因子 α（TNFα）、白细胞介素、血小板活化因子、白三烯、血栓素 A2 和补体级联的激活剂。中性粒细胞和血小板活化也可能起作用。可能参与败血症级联的其他药剂包括粘附分子、激肽、凝血酶、心肌抑制物质、β-内啡肽和热休克蛋白。内皮细胞衍生的舒张因子和内皮素-1 从内皮释放，似乎发挥调节作用相互抵消。脓毒症的中枢介质似乎并不存在，尽管 TNFα 通常被提议具有该作用。由于跨物种差异和实验设计的变化，动物研究难以推断到临床环境。脓毒症更可能与靶细胞的活化状态、附近存在的其他介质以及靶细胞释放其他介质的能力有关，而不是由任何单一致病机制引起。同样重要的是这些介质的下调或负反馈，或天然炎症抑制剂（如白细胞

介素-4 和白细胞介素-8）的产生。脓毒症中的内皮损伤可能是由持续和重复的炎症性损伤引起的。最终，这些损伤产生了足够的损坏，使下调不再发生；这导致了一种代谢无序的状态，此状态下身体无法控制自身的炎症反应。

10. **Andersen, L.W., Mackenhauer, J., Roberts, J.C., Berg, K.M., Cocchi, M.N., and Donnino, M.W. (2013). Etiology and therapeutic approach to elevated lactate levels. Mayo Clin. Proc. 88, 1127-1140.**

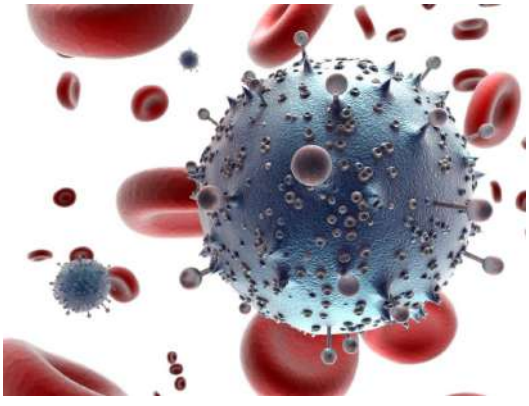
摘要

通常在急症患者中评估乳酸水平。虽然乳酸水平最常用于评估休克，但它可能因许多原因而升高。虽然组织低灌注可能是最常见的升高原因，但存在许多其他病因或促成因素。临床医生需要了解乳酸水平升高的许多潜在原因，因为乳酸水平升高的临床和预后重要性因疾病状态不同而存在很大差异。此外，可能需要根据潜在的升高原因来定制特定治疗。本综述基于对 1960 年 1 月 1 日至 2013 年 4 月 30 日期间的综合 PubMed 检索，使用搜索术语“乳酸”或“乳酸酸中毒”，结合已知的相关词汇，如：休克、败血症、心脏骤停、创伤、癫痫发作、缺血、糖尿病酮症酸中毒、硫胺素、恶性肿瘤、肝脏、毒素、过量用药和药物治疗。我们概述了乳酸水平升高的发病机制，然后深入研究了各种病因，包括与药物相关的病因。讨论了乳酸作为诊断/预后工具的优缺点及其作为临床复苏终点的潜在用途。该综述以对乳酸水平升高患者管理的一些一般性建议结束。

11. **F, B. (2000). Antithrombin III concentrates in the treatmetn of sepsis and septic shock: indictions, limits and future prospects. Minerva Anestesiol 66, 3-23.**

摘要

脓毒症和感染性休克是非心脏病重症监护病房死亡的最常见原因。尽管抗生素治疗以及血液动力学和呼吸支持方面取得了进展，但严重形式的死亡率仍然在升高。最常见的死因是多器官功能障碍综合症（MODS）。过度的炎症反应和继发于炎症和弥散性血管内凝血（DIC）的微血管床损伤是重要的致病因素。在败血症中，细胞活化的复杂系统启动活化剂和炎症抑制剂（细胞因子）的释放和相互作用，酶促级联系统的活化（凝血、纤维蛋白溶解和补体系统）以及蛋白酶和抗蛋白酶的合成。由纤维蛋白溶解系统不受控制并在微血管床中形成纤维蛋白的凝血系统的激活在 MODS 中具有重要作用。实验数据和临床观察表明抗凝血酶 III（AT）在败血症中可能具有治疗作用；脓毒症或脓毒性休克患者的血浆浓度不断降低，降低的实体与临床表现的严重程度和结果相关。具有双重功能：凝血系统的调节和抗炎特性。所述抗炎特性部分地取决于结合到糖胺聚糖的内皮细胞和前列环素的随后释放（PGI2）。抗炎作用是独立于抗凝剂的。临床使用 AT 的初步研究是在 DIC 患者组中进行的，这些患者与不同病因的病理相关并且大多病情非常危急。通常，评估标准是实验室数据的改进或标准化。由于这些研究的不完整性，对 AT 的治疗效果的解释是困难的。对死亡率的影响是有争议的。最近发表了三项前瞻性随机双盲研究，对严重脓毒症和感染性休克患者进行了研究。单项研究的结果尚无定论，但每项研究中纳入的患者数量有限，可以解释结果。对涉及严重脓毒症和败血症性休克患者的数据的荟萃分析证明奇数比率（OR）为 0.43，95%置信区间为 0.20 ~ 0.92（p = 0.029）。对第三阶段研究结果的初步分析是不确定的。治疗的时间、剂量和持续时间仍有待商榷。从视角来看，AT 可用于与止血系统激活（心脏手术、干细胞移植、烧伤）相关的其他临床病症，即使初步结果必须通过前瞻性研究证实。所有这些数据表明严重脓毒症和脓毒性休克是治疗的主要标准。

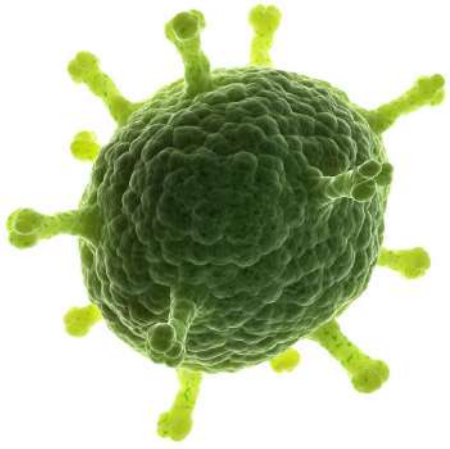


12. **R, B. (2014). Peripheral venous and arterial lactate agreement in septic patients in the emergency department: a pilot study. Eur J Emerg Med 21, 139-141.**

摘要

乳酸盐测量通常用于败血症的预后和指导治疗。尽管

静脉内乳酸盐测量已广泛使用，但大多数研究仍使用动脉乳酸盐（A-LACT）作为指标。测量之间的可互换性是值得商榷的。该试点研究旨在调查外周静脉乳酸（PV-LACT）和 A-LACT 之间是否存在关于急诊科（ED）脓毒症的协议。自 2010 年 11 月至 2011 年 8 月期间，向三级医院 ED 方便性选取 37 名患者的样本，测量 PV-LACT 乳酸盐和 A-LACT。使用 Bland-Altman 分析评估配对测量之间的一致性。测量值（静脉、动脉）之间的平均差异为 0.54 mmol/L，该协议的 95%限制区间为-0.11 ~ 1.18 mmol/L。该试验研究证明了 PV-LACT 可能用于替代脓毒性 ED 患者的 A-LACT 测量。然而，需要进一步的确定性研究以支持外周静脉乳酸的广泛临床应用。



13. D, D. (2018). Lactate - Arterial and Venous Agreement in Sepsis: a prospective observational study. Eur J Emerg Med 25, 85-91.

摘要

背景：脓毒症是急诊科（ED）的常见病。乳酸盐测量是管理的重要组成部分：动脉乳酸（A-LACT）测量是金标准。外周静脉乳酸（PV-LACT）的使用越来越多；然而，很少有研究支持两种措施的可互换性。如果 PV-LACT 与 A-LACT 具有良好的一致性，它将显著减少患者的不适和大量急性不适患者的动脉采样风险，同时也会使更快更广泛的筛查成为可能，进而可能降低医疗保健系统的成本。

目的：本研究的目的是确定 PV-LACT 和 A-LACT 在 ED 接收的脓毒症患者中的一致性。

方法：我们对一个英国国民保健署急诊室（每年 110000 名成人就诊）的 304 名同意实验的脓毒症患者进行了一项前瞻性观察性队列研究，将 PV-LACT 和 A-LACT 结果配对。进行 Bland-Altman 分析以确定一致性。构建了受试者工作特征曲线和 2 × 2 表，以探索 PV-LACT 对 A-LACT 的预测价值。

结果：平均差异（PV-LACT-A-LACT）为 0.4 mmol/L [95%置信区间（CI）：0.37 ~ 0.45]，95%的一致性限制区间为-0.4（95% CI：-0.45 ~ -0.32） ~ 1.2（95% CI：1.14 ~ 1.27）。PV-LACT 至少为 2 mmol/L，预测 A-LACT 至少为 2，灵敏度为 100%（95% CI：89% ~ 100%），特异性为 83%（95% CI：77%～87%）。

结论：这项研究是两项测量的最大比较，并显示出良好的临床一致性。我们建议在脓毒症患者的常规筛查中使用 PV-LACT。小于 2 mmol/L 的 PV-LACT 可以预测小于 2 mmol/L 的 A-LACT。

14. EE, B. (2019). Concordance of Fingerstick and Venipuncture Sampling for Fertility Hormones. Obstet Gynecol 133, 343-348.

摘要

背景：微创手指采样可以测试家族中的生殖激素水平，为女性提供更多的机会，可以筛查多囊卵巢综合征、原发性卵巢功能不全、垂体和甲状腺功能障碍等疾病。

方法：我们提出了一项测量程序比较研究，对照来自 130 名年龄在 18 ~ 40 岁的女性静脉穿刺和手指针刺样本，在月经周期第 3 天进行测试。测量样本的抗苗勒激素、雌二醇（E2）、卵泡刺激素（FSH）、黄体生成素（LH）、催乳素（PRL）、睾酮、促甲状腺激素（TSH）和游离甲状腺素（T4）水平。使用美国食品和药品监督管理局清除的免疫测定法测试样品，其中针对手指针刺样品的步骤稍加修改。

经验：静脉穿刺和手指针刺激素值均在所有测定范围内呈一致的和线性的。没有证据表明测定范围内存在系统偏差，偏差测量值低于推荐指南。对于每种激素，静脉穿刺和手指针刺之间的相关性在 0.99 ~ 1.0。每个测定显示出高度的精确度（小于 13%的变异系数）和高水平的准确度（平均回收率等于 95.5%～102.3%）。

结论：静脉穿刺和手指针刺样本可以互换使用，以测量抗苗勒激素、E2、FSH、LH、PRL、睾酮、TSH 和游离 T4

水平。手指针刺采样为医生和女性提供了更方便的测试选择。

资金来源：该研究由 Modern Fertility 赞助。

15. **Edelman, A. (2007). A comparison of blood spot vs. plasma analysis of gonadotropin and ovarian steroid hormone levels in reproductive-age women. Fertil Steril 88, 1404-1407.**

摘要

目的：比较从血斑与血浆（单次访视研究）获得的 LH、FSH、P 和 E_2 水平，并确定血斑是否能记录排卵周期中的循环激素水平（月经周期研究）。

设计：横断面研究。

单位：学术中心。

患者（S）：18 ~ 35 岁的女性，月经周期正常，最近没有使用激素避孕药。

干预（S）：女性在月经周期的随机日（n = 100，单次研究访问），通过静脉穿刺提供血斑样本，通过手指穿刺提供血浆样本。另外 5 名女性进行了整个月经周期，每周两次静脉穿刺和每日自我采集的血斑取样。分析样品的 FSH、LH、P 和 E_2。

主要观察指标（S）：血斑和血浆水平之间的相关性。

结果（S）：单次就诊研究中血斑和血浆样本之间存在显著的正相关 [r^2：FSH，0.91；LH，0.93；P，0.83；和 E_2，0.70]。5 名月经周期研究女性中有 2 名基于 P 水平（ > 3 ng/mL）和 LH 激增进行了排卵周期。每日血斑采样比双周静脉穿刺更能记录荷尔蒙变化。

结论（S）：FSH、LH、P 和（在较小程度上）E_2 的血斑监测似乎与用于临床研究和护理的传统血浆测定一样有效。

16. **Pollock, N.R., Rolland, J.P., Kumar, S., Beattie, P.D., Jain, S., Noubary, F., Wong, V.L., Pohlmann, R.A., Ryan, U.S., and Whitesides, G.M. (2012). A paper-based multiplexed transaminase test for low-cost, point-of-care liver function testing. Sci. Transl. Med. 4, 152ra129.**

摘要

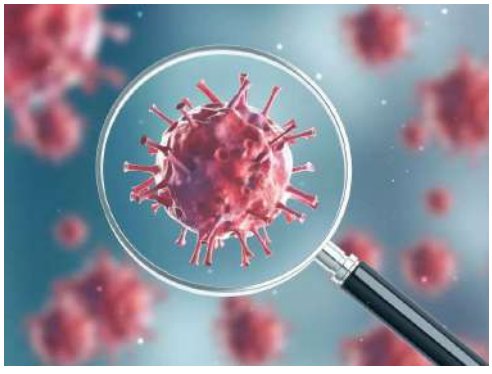
在发达国家，通过连续测量高危个体的血清转氨酶 [天冬氨酸氨基转移酶（AST）和丙氨酸氨基转移酶（ALT）] 来监测药物引起的肝损伤是标准治疗方法。尽管有这种需要，但在资源有限的环境中监测与药物相关的肝毒性往往受到费用和后勤的限制，即使对于风险最高的患者也是如此。本文介绍了基于纸膜的多重微流体检测方法的开发和临床测试，该检测方法用于快速、半定量测量手指针刺样品中的 AST 和 AL。使用通过静脉穿刺获得的 223 个临床标本和来自健康志愿者的 10 个手指针刺标本，我们已经证明我们的测定可以在 15 分钟内提供全血或血清中 AST 和 ALT 的视觉测量，这允许用户将这些值作为三个读出“箱”[< 3× 正常上限（ULN），3 ~ 5 × ULN，和 > 5 × ULN，对应于结核 /HIV 治疗指南] 中之一，准确度 > 90%。这些数据表明，最终的即时诊断——指尖采血装置将在资源匮乏的环境中对患者护理产生巨大影响。

17. **Pollock, N.R., McGray, S., Colby, D.J., Noubary, F., Nguyen, H., Nguyen, T.A., Khormaee, S., Jain, S., Hawkins, K., Kumar, S., et al. (2013). Field evaluation of a prototype paper-based point-of-care fingerstick transaminase test. PLoS ONE 8, e75616.**

摘要

通过对潜在肝毒性药物（例如，HIV 和肺结核）患者进行连续转氨酶测量来监测药物诱导的肝损伤（DILI）在资源丰富的国家是常规的，但在资源有限的环境中通常无法进行。为了使人们能够普遍获得对 DILI 的负担得起的即时诊断（POC）筛查，我们对纸质微流体指尖试验进行了首次现场评估，以快速、半定量、可视化测量血液丙氨酸氨基转移酶（ALT）。我们的目标是评估操作可行性、操作员间可变性、批次可变性，设备故障率

和准确性，以通知设备修改以进行进一步的现场测试。对来自越南 POC 上接受 HIV 治疗的 600 名门诊病人的指尖样本进行基于纸质的 ALT 测试。将两名门诊护士独立阅读的结果与平行获得的静脉穿刺样本的金标自动化（Roche Cobas）结果进行比较。依次使用两个设备批次。我们展示了高度的运营商间协议，96.3%（95% CI，94.3% ~ 97.7%）将视觉结果放入临床定义的“箱”（< 3x，3 ~ 5x 和 > 5x 正常上限），> 90%有效性确定一致，组内相关系数为 0.89（95% CI，0.87 ~ 0.91）。由于溶血，在百分比中观察到批次变异性（第 1 批为 21.1%，第 2 批为 1.6%）并且与许多掺入的血浆分离膜相关。所有其他设备控件均观察到无效率 < 1%。两位读者的整体装箱准确率为 84%（84.3%/83.6%）。我们的研究结果表明，在目标临床环境下，由当地医生进行的操作人员对视觉阅读的一致性极高，这表明设备操作和阅读过程是可行的和可重复的。料箱放置精度和批次间变异性数据确定了设备优化和材料质量控制的特定目标。这是用图案化的纸基微流体装置进行的第一次现场研究，为其他重要分析物的类似分析开发了大门。



18. **Pollock, N.R., Colby, D., and Rolland, J.P. (2013). A point-of-care paper-based fingerstick transaminase test: toward low-cost “lab-on-a-chip” technology for the developing world. Clin. Gastroenterol. Hepatol. 11, 478-482.**

摘要

目前人们迫切需要高质量、低成本的即时诊断，这些诊断可以在资源有限的环境中使患者受益，相应地，人们对基于纸张的微流体平台的诊断实用性的兴趣也在增加。我们描述了一种新型纸质多重微流体检测方法的开发、早期临床测试和潜在临床影响，该检测设计用于手指针刺样品中天冬氨酸氨基转移酶和丙氨酸氨基转移酶的快速、半定量测量。该装置最终有望在资源有限的环境中为药物诱导的肝损伤提供经济实惠的临床即时诊断筛查，并为其他重要分析物开发类似的即时临床试验打开了大门。

19. **Jain, S., Rajasingham, R., Noubary, F., Coonahan, E., Schoeplein, R., Baden, R., Curry, M., Afdhal, N., Kumar, S., and Pollock, N.R. (2015). Performance of an Optimized Paper-Based Test for Rapid Visual Measurement of Alanine Aminotransferase (ALT) in Fingerstick and Venipuncture Samples. PLoS ONE 10, e0128118.**

摘要

背景：开发了一种基于纸张的多重微流体检测方法，用于在手指针刺样品中目测测量丙氨酸氨基转移酶（ALT），从而产生快速、半定量的结果。前期研究表明该方法准确性有待提高；随后使用 FDA 批准的自动化平台（Abaxis Piccolo Xpress）作为对照优化该装置。在此，与 Abaxis 和 Roche/Hitachi 平台相比，我们评估了针对手指针刺血液和血清中 ALT 测量的优化纸张测试的性能。为了评估远程结果解释的可行性，我们还将完成测试的手机摄像头图像与实时读取设备进行了比较。

方法：96 名不同基线 ALT 浓度的门诊患者使用纸质装置进行手指针刺试验；完成设备拍摄手机图像并将其发送给非现场盲阅读器。静脉穿刺血清来自 93/96 参与者进行常规临床试验（Roche/Hitachi）；随后，采集 88/93 血清样品并应用于纸和 Abaxis 平台。通过 Bland-Altman 分析比较纸张测试和参考标准结果。

结果：对于血清，纸质测试和 Abaxis 结果之间存在极好的一致性，偏差可忽略不计（+4.5 U/L）。Abaxis 结果比 Roche/Hitachi 结果系统地低 8.6%。在纸上测试的手指针刺样品中的 ALT 值系统地低于在纸上测试的配对血清中的值（偏差-23.6 U/L）或 Abaxis（偏差-18.4 U/L）；为纸张装置开发了校正因子，以将手指针刺血液与血清相匹配。手机图像的视觉读取与实时读取（偏差+5.5U/L）非常匹配。

结论：纸质 ALT 测试对于血清测试是高度准确的，与参考方法相匹配，优化的参考方法优于彼此匹配的参考方法。手指针刺和血清样本中的 ALT 值相比，之间存在系统差异，并且可以通过对手指针刺值应用校正因子来解决。远程读取此设备是可行的。

20. S, H. (2013). Muscle fatigue experienced during maximal eccentric exercise is predictive of the plasma creatine kinase (CK) response. Scand J Med Sci Sports 23, 501-507.

摘要

习惯的离心运动可能会导致骨骼肌损伤，血浆肌酸激酶（CK）活动增加。虽然已经很好地描述了 CK 对标准化延长收缩的反应中个体的广泛差异，但是尚未理解这种现象背后的原因。因此，本研究调查了离心运动后肌肉损伤间接标志物的变化与运动期间肌肉性能下降的可能相关性。27 名健康未训练的男性受试者进行了三组 30 次膝关节伸肌的最大等速离心收缩。使用等速测力计记录肌肉工作，以通过各种疲劳指数评估肌肉疲劳。在运动前（运动前）和运动后一天（运动后）测量血浆 CK 活性，肌肉酸痛和僵硬度。离心运动引起三种肌肉损伤间接标志物的显著变化。对于所有测量标准，观察到大的受试者间变异性。更有趣的是，log（CK 后/CK 前）和肌肉僵硬似乎与相对工作减少密切相关（$r = 0.84$，$r^2 = 0.70$ 和 $r = 0.75$，$r^2 = 0.56$）。这是第一项提出最大偏心方案期间肌肉疲劳曲线可以预测与人类肌肉损伤相关的症状程度研究。

21. P, B. (2007). Creatine kinase monitoring in sport medicine. Br Med Bull 81-82, 209-230.

摘要

普遍一致的领域：总肌酸激酶（CK）水平取决于年龄、性别、种族、肌肉质量、体力活动和气候条件。在明显健康的受试者中，血清 CK 的高水平可能与体能训练状态有关，因为它们依赖于肌瘤损伤：剧烈运动损伤骨骼肌细胞导致血清 CK 总量增加。运动后血清酶活性最高的是长时间运动后，如超距离马拉松或负重运动和下坡跑，其中包括偏心肌肉收缩。运动后 24 小时血清 CK 总活性显著升高，患者休息后逐渐恢复到基础水平。持续升高的血清 CK 水平偶尔出现在健康的人，也显著增加在肌肉疾病的临床前阶段。

有争议的领域：一些作者在研究休息时 CK 水平较高的受试者时发现，数年后，受试者出现肌肉无力，并提出早期肌病可能无症状。另一些研究表明，在这些患者中，高血钙症可能并不意味着疾病。在许多情况下，由于症状只有在运动后才会显现出来，因此不能在患者休息时进行常规检查后制定诊断。一些作者认为力量训练对肌病患者似乎是安全的，尽管常规运动处方的证据仍然不足。另一些人认为，在这种情况下，剧烈的长时间运动可能会产生负面影响，因为在肌肉蛋白质不断减少的情况下，长时间运动不会导致肌肉对体育锻炼的生理适应。

生长点：运动员在绝对休息后，在没有任何其他诱发因素的情况下，血清 CK 水平较高，应促使对肌肉无力或其他简单症状进行全面诊断检查，这些症状在运动员和久坐不动的受试者中并不总是立即明显。这些迹象可能表明亚临床肌肉疾病，训练负荷可能通过深度疲劳的开始证明。建议怀疑患有肌病的运动员继续以较低的强度进行体育活动，以防止高强度运动造成的肌肉损伤，并使充分的恢复有利于充分的恢复，这可能是安全的。

及时开展研究的领域：CK 值在个体之间表现出很大的变异性。一些运动员对体育训练反应较低，长期血清 CK 水平较低。部分运动员反应灵敏，酶值较高，训练水平、肌肉大小、纤维类型与运动后 CK 释放的关系有待进一步研究。此外，通过对运动后 CK 水平较高的健康运动员应激后 CK 动力学的评价，将结果与静止状态下持续性高钾血症运动员的结果进行比较，可以得到更多关于高钾血症的细节。最后，量化哪种运动更适合患有肌病的运动员，以及运动强度对病理进展无危险，这一点很重要。

22. MA, K. (2010). Capillary and venous samples of total creatine kinase are similar after eccentric exercise. J Strength Cond Res 24, 3471-3475.

摘要

循环肌酸激酶（CK）水平经常被作为抗阻力运动后肌肉损伤的一种间接生物标志物被监测。本研究的目的是评估末梢血取样，一种获得静脉血样本的更简单、更少侵入性的方法，是否能够比静脉穿刺更可靠地测量总

CK。15 名未经训练的受试者进行了 50 次最大偏心肘关节伸展，以诱导肱二头肌的肌肉损伤。在运动后 24 小时、48 小时、72 小时和 96 小时同时采集末梢管（指尖）和静脉全血样本。使用一种商业 CK 分析试剂盒，并对标准进行修改，以减少样品量，通过分光光度法同时分析静脉血和末梢血样品的总 CK 活性。结果表明，总 CK 的采样点之间的相关性为 0.997，而在 CK 值范围内，静脉血和，末梢血样本之间的差异估计为 < 12%。这些发现表明，对总 CK 活性的末梢血取样是静脉穿刺的有效替代方法，在间接评估运动后肌肉损伤时，研究人员、临床医生、力量和条件反射专家应将其作为替代取样技术加以考虑。

23. de Oliveira, D.C.X., Frisselli, A., de Souza, E.G., Stanganelli, L.C.R., and Deminice, R. (2018). Venous versus capillary sampling for total creatine kinase assay: Effects of a simulated football match. PLoS ONE 13, e0204238.

摘要

背景：与静脉穿刺相比，末梢穿刺可能是一种更简单且侵入性更小的血液采集方案，其将增加潜在采样测试的数量。然而，如果可以使用末梢血采样作为静脉穿刺的替代方案来确定血浆的变化，则对足球训练期间的总肌酸激酶（CK）活性知之甚少。

目的：本研究旨在确定末梢血采样是否能提供与静脉穿刺相比的总 CK 活动的代表性测量，以应对足球训练期间诱导的 CK 血浆水平升高。

方法：来自 19 岁以下足球队的 22 名球员进行模拟足球比赛，每队有 11 名球员，总持续时间为 90 分钟（两半，45 分钟，休息 15 分钟）。在训练期间（24 h 和 48 h）之前和之后收集静脉血和耳垂末梢血样品。在恢复期间，运动员在运动后连续三天重新测试。

结果：在静脉血（1.7 倍）和末梢血（1.9 倍）采血中测定的模拟匹显著增加（$p < 0.05$）总 CK 活性。与静脉血总 CK 相比，使用末梢血测定的总 CK 活性表现出显著的相关性（r = 0.85；$p < 0.01$）和升高的一致性 Lin 指数（pc = 0.80）。Bland-Altman 图显示末梢血 CK 高估了静脉血 CK 水平 130 U/L（61%），具有缓和方差和低偏差。

结论：我们的研究结果表明，总 CK 反应的末梢血采样可被认为是静脉穿刺的可靠替代方案，可用于确定足球训练期间血浆总 CK 活性的变化。

24. Fuller, B.M., and Dellinger, R.P. (2012). Lactate as a hemodynamic marker in the critically ill. Curr. Opin. Crit. Care 18, 267-272.

摘要

回顾的目的：早期的定量复苏策略可改善重症患者的预后。这种策略的血液动力学终点一直是文献中争论的话题。本综述着重于使用乳酸作为风险分层的标记，乳酸清除作为血液动力学终点，并将其与混合静脉氧合作为复苏目标进行比较。

最新发现：乳酸清除率与几个重症患者队列的改善结果相关。乳酸水平和中心静脉血氧饱和度经常是不一致的。作为定量复苏策略的一部分，靶向乳酸清除可能与靶向中心静脉血氧饱和度一样有效。

总结：重症患者的复苏应针对组织缺氧的逆转。使用乳酸作为血液动力学标记物和复苏终点具有生理意义，并且受到最近数据的支持。乳酸清除剂与其他传统复苏终点（如混合静脉血氧饱和度）的使用应基于个体患者的临床特征和反应。

25. Bakker, J., Nijsten, M.W., and Jansen, T.C. (2013). Clinical use of lactate monitoring in critically ill patients. Ann. Intensive Care 3, 12.

摘要

危重病人常见血乳酸水平升高（高乳血症）。尽管经常用于诊断组织氧合不足，但与组织氧合无关的其他过程可能会增加乳酸水平。特别是在重症患者中，糖酵解增加可能是高乳血症的重要原因。然而，乳酸水平升高的存在对高乳血症患者的发病率和死亡率具有重要意义。虽然经常使用术语乳酸性酸中毒，但乳酸和 pH 之间的显著关系仅存在于较高的乳酸水平。因此，术语乳酸相

关的酸中毒更合适。最近的两项研究强调了监测乳酸水平和调整治疗早期复苏中乳酸水平变化的重要性。由于可以在床边从各种来源快速测量乳酸水平，因此应将结构化乳酸盐测量结合到复苏方案中。

26. J, C. (2015). Effectiveness of arterial, venous, and capillary blood lactate as a sepsis triage tool in ED patients. Am J Emerg Med 33, 167-172.

摘要

目的：评价动脉（ABL）、周围静脉（VBL）和毛细血管（CBL）血乳酸浓度对脓毒症患者早期发现严重脓毒症的能力。

方法：对急诊就诊的脓毒症患者进行前瞻性研究。使用手持式床旁分析仪在动脉、外周静脉和毛细血管血液的微量样品测量血乳酸。将动脉血样送至中心实验室作为参考测量。

结果：本研究共纳入 103 例患者，其中严重脓毒症 63 例。护理点与血乳酸参考值之间存在较强的相关性。CBL、VBL 和 ABL 也都存在显著差异（分别为 3.01 ± 0.29、2.51 ± 0.21、2.03 ± 0.18 mmol/L；$p < 0.001$）。VBL 值对早期发现严重脓毒症最有效（VBL、ABL、CBL 在受体工作特征曲线下面积分别为 0.85 ± 0.04、0.76 ± 0.05、0.75 ± 0.05；$p < 0.01$）。28 天死亡率与脓毒症的严重程度（28.6% vs. 7.5%）和器官功能障碍（$p < 0.01$）有关。动脉血乳酸、VBL、CBL 均与第 28 天死亡率显著相关。

结论：初始 VBL 可有效评估脓毒症的严重程度，甚至比 ABL 和 CBL 更能早期发现严重脓毒症的存在。

27. D, D. (2017). Capillary and Venous Lactate Agreement: a pilot prospective observational study. Emerg Med J 34, 195-197.

摘要

背景：血乳酸是患者疾病严重程度的标志。乳酸末梢血（CAP-LACT）测量可以潜在地改善患者筛查；然而，它的临床效用证据不足。

目的：我们的目的是研究 CAP-LACT 与外周静脉乳酸（PV-LACT）之间的一致性。

方法：我们对 99 名需要乳酸盐测量的患者进行了一项前瞻性观察性试验研究。记录配对的 CAP-LACT 和 PV-LACT。一致性由 Bland-Altman 分析确定。

结果：偏差为 0.2 mmol/L，95%的一致性限值为-1.9 ~ 2.3。

结论：CAP-LACT 与 PV-LACT 的一致性较差。需要进一步研究以提高其潜在的临床效用。

28. D, L. (2013). Assessment of using fingerstick blood sample with i-STAT point-of-care device for cardiac troponin I assay. Am J Emerg Med 31, 1236-1239.

摘要

目的：本研究的目的是比较心肌肌钙蛋白 I 的末梢血采血护理点（POC）检测与使用 i-STAT 设备的常规静脉穿刺 POC 测试。

基本程序：本研究于 2011 年 6 ~ 8 月在郊区一级创伤中心急诊部（ED）进行，获得了机构审查委员会的批准。从自愿接受静脉穿刺标准 POC 肌钙蛋白（POCT）检测的患者身上采集末梢血样作为检查的一部分。心肌肌钙蛋白 I（cTnI）检测使用 I-stat 1 装置（雅培护理点，普林斯顿，新泽西州）。采用 SAS 9.2 软件（SAS、Cary、NC）对数据进行分类比较、线性回归、Bland-Altman 一致性分析。

主要结果：通过末梢血和标准静脉穿刺 ED POC 测试测量了 89 个 cTnI 水平。其中 4 个出现墨盒错误，其余 85 人进行了分析。与标准 ED POCT 相比，末梢血检测的阳性预测值为 1.00（0.48，1.00），阴性预测值为 0.96（0.89，0.99），灵敏度为 0.625（0.24，0.91），特异性为 1.00（0.95，1.00）。方法之间的关系呈线性关系，线性回归方程 ED POCT 水平 = 0.0062 + 1.3752 × 末梢血水平（$p < 0.0001$）。Bland-Altman 一致性分析得出末梢血与 ED POCT 之间的平均差异为-0.0095，一致性限制为-0.0625 ~ 0.0435。

主要结论：如果不用矫正项，使用 i-STAT 测定末梢血 cTnI 不足以确定肌钙蛋白的确切水平。然而，末梢血测试在将肌钙蛋白水平定义为阴性、临界或阳性时是准确的，因此能够提供可指导诊断和治疗决策的临床信息。

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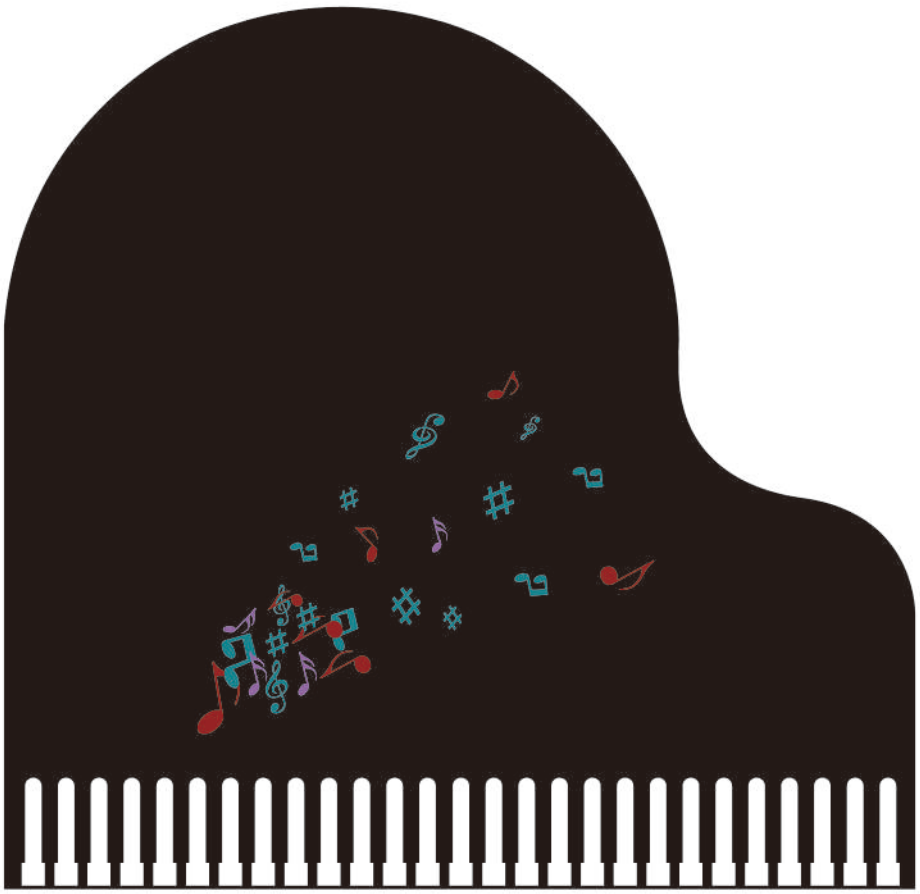

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文献精读

本期的文献精选是一篇专业文章《一种优化的试纸条方法快速视觉测量手指末梢血和静脉血丙氨酸氨基转移酶（ALT）的性能研究》，原文标题为“Performance of an Optimized Paper-Based Test for Rapid Visual Measurement of Alanine Aminotransferase (ALT) in Fingerstick and Venipuncture Samples”。这是来自美国波士顿的多个团队的成功合作，使用的检出平台是一种新型的基于纸质介质的多重微流体测定法，有快速、半定量的特点。这是一个即时检测的方法学，末梢血为首选样本，而末梢血和静脉血样本是否等效就成为关键的话题。在研究中对 96 名具有不同基线 ALT 浓度的门诊患者使用纸质装置进行手指针刺试验，而静脉血来自 93/96 参与者进行常规临床试验（Roche/Hitachi）。研究数据表明手指末梢血和静脉血样本中的 ALT 值之间存在系统差异，并且可以通过对手指末梢血样本的值应用校正因子来解决。

一种优化的试纸条方法快速视觉测量手指末梢血和静脉血丙氨酸氨基转移酶（ALT）的性能研究

摘要

背景：基于试纸条的多重微流体测定法用于在末梢血样品中可视地测量丙氨酸氨基转移酶（ALT），并快速得到半定量结果。之前研究表明需要提高准确性，随后使用 FDA 批准的自动化平台（Abaxis Piccolo Xpress）作为对比。与 Abaxis 和 Roche/Hitachi 平台相比，我们评估了针对末梢血和血清中 ALT 试纸条测试的性能。为了评估远程结果解释的可行性，我们还将完整测试的手机摄像头图像与实时读取设备进行了比较。

方法：96 名具有不同基线 ALT 浓度的门诊患者使用试纸条进行末梢血试验，拍摄完整设备的手机图像并将其发送至盲选的非现场阅读器。从 96 个参与者采集 93 个人的静脉血清进行常规临床试验（Roche/Hitachi），随后，其中 99 份血清样本用试纸条和 Abaxis 平台检测。试纸条检测和参考标准结果通过 Bland-Altman 分析进行比较。

结果：血清的试纸条和 Abaxis 检测结果之间存在极好的一致性，偏差可以忽略不计（+4.5 U/L）。 Abaxis 结果比 Roche/Hitachi 结果系统地低 8.6%。试纸条检测的末梢血 ALT 值系统地低于试纸条（偏差-23.6 U/L）或 Abaxis（偏差-18.4 U/L）检测的血清 ALT 值。为试纸条检测方法开发了校正因子，使得末梢血与血清相匹配。手机图像的视觉读取与实时读取（偏差+5.5 U/L）非常匹配。

结论：试纸条 ALT 测试对于血清测试是高度准确的，与参考方法相匹配，优化的参考方法优于彼此匹配的参考方法。末梢血和血清样本中的 ALT 值之间存在系统差异，可以通过校正因子对末梢血进行校验。远程读取此设备是可行的。

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介绍

在资源受限的环境中，床旁（POC）诊断尤其适用于诊断和医疗管理，但对资源丰富的集中式实验室来说可能不适用或成本过高。如果可以进行集中化检测，长时间的结果周转时间

可能导致患者失去联系，从而对结果产生不利影响。考虑到这些障碍，最近 POC 诊断的开发出现了许多应用[1-3]。

需求 POC 检测的一个例子是监测转氨酶用于诊断和管理药物诱导的肝损伤（DILI）。对于治疗 HIV 和 TB 的药物，人们特别需要转氨酶检测，因为众所周知这些药物具有肝毒性[4,5]。转氨酶监测对于评估具有潜在肝脏疾病（如乙型肝炎或丙型肝炎）的患者也是有价值的。这些疾病在资源有限的情况下不成比例地影响那些疾病，并且在许多这些环境中转氨酶检测有限或无法获得，患者有增加 DILI 并发症的风险。通常，转氨酶监测需要静脉采血的器械，训练有素的抽血者，离心以分离血清或血浆，以及在大型自动化平台上进行测试。这些平台价格昂贵，需要训练有素的技术人员进行检测和维护，这使得它们在许多发展中国家的使用和扩大规模都不切实际。由于这些障碍，在许多资源有限的环境中，患有潜在肝毒性药物的患者在治疗期间接受极少或不接受监测。已开发出用于 POC 转氨酶检测的自动化平台（Roche Reflotron 和 Alere Cholestech），然而，Choletech ALT 测试目前已在全球上市，而 Reflotron 目前已在美国上市。我们最近开发了一种基于试纸条的多重微流体检测方法，用于视觉半定量测量末梢血丙氨酸氨基转移酶（ALT）[6-8]。该设备基于“图案纸”技术，其中蜡基打印机用于产生一系列疏水屏障和亲水通道，其引导流体在横向和垂直方向上通过纸张芯吸。当流体定向流过图案纸层时，它接触区域特异性测定试剂，允许在单个样品上平行进行多个反应。我们的检测方法旨在快速得到半定量的视觉结果（约 18 ~ 30 秒）；此外，它便携、一次性、不需要电源，预计成本约为 0.10 美元/次，这使其成为资源有限医疗机构的理想选择。基于试纸条的类似微流体技术正在被广泛应用，包括（例如）葡萄糖和蛋白质测量、细菌检测、丙型肝炎抗体检测、癌症生物标志物检测和血型分类[9-15]。据我们所知，在这一新兴的基于试纸条的微流体平台中，我们基于试纸条的 ALT 测试目前最接近实际的临床应用。

临床血清和全血标本的早期临床前测试表明，基于试纸条的设备可以产生 > 90%准确度的视觉测量[6]，因此可以进行现场测试。此后，在越南一家繁忙的艾滋病诊所接受护理的 600 名艾滋病病毒感染者进行了第一次末梢血评估[7]，被认为是应用该测试的理想目标设定。该评估研究表明，在该目标设置中，设备操作和读取过程既可行又极其可重复，但强调需要进一步优化设备以降低溶血率并提高准确性。该设备随后进行了广泛的优化，包括采购新的血浆分离膜，用抗溶血涂层处理该膜以降低溶血率，重新测定化学分析，并根据自动参考标准 Abaxis Piccolo 进行重新校准[16]。

我们在肝病患者或肝毒性药物治疗门诊患者中进行优化 ALT 检测的验证研究结果表明，每个人都需要 ALT 监测作为常规治疗的一部分。本研究的目的是评估一种优化的基于试纸条方法检测的性能，以测量具有一系列基线 ALT 水平患者的末梢血和静脉血清中的 ALT 水平，并与两个 FDA 批准的临床使用广泛的自动化平台血清结果相比较。因此，我们能够评估样本类型对试纸条方法测试结果的影响，以及比较试纸条方法测试的性能。

材料和方法

设备设计、生产和贮存

“图案化试纸条”ALT 测试采用蜡基印刷技术；打印后，将纸层加热至 110°C，使蜡熔化并使其透过纸张厚度（GE 沃特曼 1 级色谱纸），形成疏水环绕的微流体和亲水检测区域蜡障[17-20]。通过堆叠两个这样的图案化纸层以及血浆分离膜盘（Primecare NX 膜）和层压膜来构建 ALT 测试，并设计 3D 装置（图 1A）。采用防止样品蒸发的覆盖膜（2MIL 低密度聚乙烯，Warp Bros，Chicago IL），该覆盖膜适用于末梢血测试，不适用于使用血清测试。使用图案化的压敏粘合剂膜（Flexcon）将各层粘附在一起。血浆分离膜分离末梢血血浆中的红细胞和白细胞，使血浆能够吸收到检测区域。用抗溶血涂层处理血浆分离膜以防止细胞在过滤期间溶血。

ALT 试验利用基于过氧化物酶的比色测定法[6]，在 ALT 升高的情况下产生红色。测试点的红色强度与样品中 ALT 的浓度成正比，通过与参考颜色图表或“阅读指南”（图 1B）的比较，可以对结果进行半定量，可视化的解释。“阅读指南”还允许用户将 ALT 结果置于三个临床相关区域之一 [< 3X 正常上限（ULN），3-5X ULN 和 > 5X ULN] 用于管理有 DILI 风险的患者。阴性对照点可作为样品充分性的指标（适当的样品体积，无溶血），阳性对照斑点可作为测试时试剂活性的指标（图 1C 和 1E）。在该测定中，和任何其他酶促反应一样，反应速率是温度依赖性的。因此，使用温度/读取时间图表确定必须读取测试的时间窗口（图 1D）。每个控制区域都被解释为“有效”或“无效”。任一控制区上的“INVALID”都会使设备无效[7]（图 1E）。

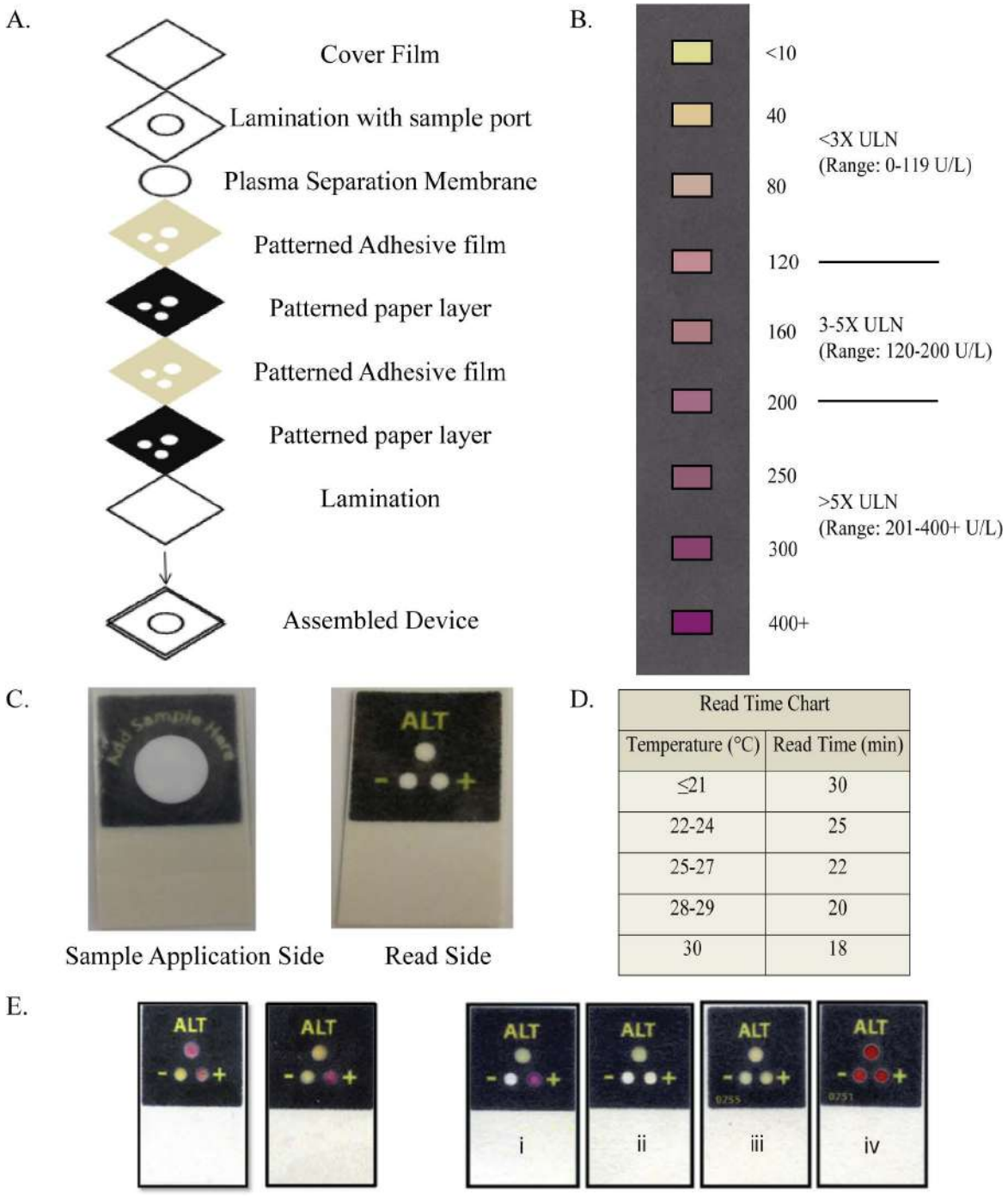


图 1. 3 区 DFA 试纸条丙氨酸氨基转移酶（ALT）测试示意图。**A.** 通过组装两个图案化纸层，等离子体分离膜盘和保护性层压膜来构建 DFA ALT 测试以产生三维装置。将覆盖膜应用于与末梢血样品一起使用的装置；该覆盖膜不适用于与血清样品一起使用的装置。使用图案化的压敏粘合剂膜将所有层粘附在一起。**B.** 该测试利用基于过氧化物酶的比色测定，通过与参考颜色图表的视觉比较，确定半定量 ALT 结果。颜色图表还允许用户将 ALT 结果放入三个分类区间中的一个：< 3X 正常上限（ULN）、3 ~ 5X ULN 和 > 5X ULN。**C.** 将末梢血样品施加到“样品施加侧”上的样品端口。通过血浆分离膜分离血细胞，允许血浆芯吸到装置中并与各个检测区域的试剂反应；在“读取侧”观察结果。**D.** 在对应于环境温度的孵育时间之后读取结果。**E.** 测试中的两个控制区用于确定测试结果的有效性。有效设备的示例显示在左侧的两个图像中。右侧显示了四个无效示例，如下所示：**i.** 由于阴性对照区缺少黄色表明样品体积不足。**ii.** 样品体积不足（如 i）和阳性对照失败，后者表明在测试时无活性试剂。**iii.** 积极控制失败。**iv.** 阴性对照区中存在红色表示溶血样品。

使用已有技术[6,7]在全民诊断公司（DFA）中并行制造了400 台设备。试纸条测试通过三步反应中的酶活性测量 ALT。测试区在第一层中含有 L-丙氨酸和 α-酮戊二酸（作为底物），并且样品中存在的 ALT 催化氨基从 L-丙氨酸转移至产生丙酮酸和谷氨酸的 α-酮戊二酸。在第二步中，包含在 ALT 测试区第二层中的丙酮酸氧化酶将丙酮酸氧化成乙酰磷酸盐和过氧化氢。在反应的最后步骤中，辣根过氧化物酶（HRP）催化过氧化氢与 4-氨基安替比林和间甲苯基二乙醇胺（TDA）的反应，产生红色/粉红色染料复合物，其与样品中的 ALT 浓度相关。该酶促反应类似于在其他 ALT 测试中使用的酶反应，特别是 Cholestech LDX 和 Reflotron 系统。

化学反应如下：

与依赖于以前 DFA 纸 ALT 试验[6-8]中使用的 4-氨基安替比林和 3,5-二氨基苯甲酸（DABA）配方相比，该配方产生更强的颜色变化和更大的线性动态范围。在优化的试纸条测试中，丙氨酸和 α-酮戊二酸在与剩余试剂分开层中的点样改善了试纸条测试的稳定性和性能。向所有试剂中加入稳定剂（葡萄糖血清白蛋白）以延长保质期。加速稳定性研究表明，当在推荐的温度范围（18 ~ 30℃）下储存时，该装置可稳定约 18 个月。

将这些装置单独装入箔衬里的铝袋中，每个袋装有干燥剂，并在室温下储存一段时间。根据 DFA 的标准操作程序，设备在发布之前经过质量测试，并用于本研究。

内部精确研究

在目标浓度（低、中和高）下用纯化的 ALT（LeeBio 溶液）掺加正常人血清（Valley Biomedical）制备三种标准品。运行精密度研究的操作员对这些样品不知情。这三个标准分别在 Hepatic Function Panel 和 Abaxis Piccolo Xpress 上进行了五次重复测试[16]，并在同一天的 DFA 试纸条中重复测试 10 次。对 Abaxis Piccolo 和 DFA 试纸条测试计算%变异系数（CV）。这项精确研究分别在 Abaxis Piccolo、Hepatic Function Panel 和 DFA 试纸条设备上进行。

毛细管吸样量准确性

在临床研究中使用 35 µL Microsafe 毛细管（Safe-Tec）采集末梢血样品。使用新采集的全血（Research Blood Components LLC）和去离子水测试毛细管吸样体积准确度。使用校准的分析天平（NewClassic MF，Mettler Toledo，Columbus OH）测量分配体积的重量。计算出去离子水和全血的%CV。

道德声明

该研究得到了 Beth Israel Deaconess 医学中心（BIDMC）机构审查委员会的批准。IRB 批准使用口头（而非书面）同意进行这项最小风险研究；每个人都阅读了描述研究目的和程序的口头同意书，获得并记录了口头同意，并为受试者提供了研究信息表。

参与者

参与者从 BIDMC 肝脏中心和传染病门诊诊所招募。为了参与研究，参与者必须在每天的临床常规中接受 ALT 测试（采集静脉穿刺血液，然后在 Roche/Hitachi 平台上进行测试）。招募是基于以前的 ALT 结果，以便在整个临床范围内包括 ALT 值的患者；先前 ALT ≥ 120 U/L 的患者在可能的情况下优先进行招募。根据上述选择标准连续对参与者进行抽样，并获得口头知情同意书。通过图表审查采集每个参与者的数据，包括基本的人口统计信息、最近的实验室测试结果（包括登记当天的 ALT 水平）和肝病的主要原因（如果已知）。

测试程序

操作者 1 使用 Surgilance SLN300 采血针（Medipurpose）采集参与者末梢血。对于前 42 位患者，[使用 35 µL Microsafe 毛细管（Safe-Tec）] 采集第一滴末梢血并滴到装置上。接下来的 54 名患者，擦去第一滴血液，用毛细管采集 35 µL 样品并滴到装置上，上样后启动计时器。采集末梢血后，对患者进行静脉采血做常规临床 ALT 检查。操作者 1 记录了获得所需样本量和末梢血采集的温度/相对湿度的难度（如果有的

话）。将该装置置于培养皿中并将其（与计时器和研究表一起）带到孵育室，操作员 2（对任何先前患者 ALT 值不知情）解释并记录结果。操作者 2 基于培养室的温度选择读取时间。结果以 U/L 报告（四舍五入到最接近的 10 U/L）。操作员 2 还通过目视检查对照区域的溶血、不完全填充和试剂功能来确认该装置的有效性。记录了孵育室温度和相对湿度。

在读取设备结果后，操作员 2 拍摄设备并对比“阅读指南”，以便远程读取测试图像。这些照片是使用两部手机拍摄的，一部带有 800 万像素摄像头（三星 Galaxy SII），另一部带有 200 万像素摄像头（AT&T Z431）。两组照相手机图像都发送到两个单独的电子邮件地址。两个独立的操作员（操作员 3 和 4）使用在标准笔记本电脑显示器上的图像中捕获的“阅读指南”（操作员 3 从两百万像素摄像头读取图像，操作员 4 从八百万像素摄像头读取图像）读取两个图像集。操作员 3 和 4 都对操作员 2 注意到的结果不知情。

根据临床常规，BIDMC 临床实验室（血清，Roche/Hitachi 平台，无磷酸吡哆醛激活[21]）采集静脉血进行 ALT 测试。将临床血清样品在 4℃下保持 5 天，然后识别在 DFA 试纸条装置和 Abaxis Piccolo 分析仪上测试[16]。在 DFA 试纸条装置上进行的测试由另一个受过训练的操作者进行，该操作者不知道 Piccolo 结果和研究中收集的所有其他结果。

统计分析

通过图形方式、平均差异或偏差以及 95%的一致性评估测量方法之间的一致性。图表包括“对角线图”（其中一种方法简单地相对于另一种方法绘制并相对于平等线进行评估）和 Bland-Altman 图[22]。对于 Bland-Altman 分析，使用对数变换进行比较，其中差异的平均值和标准偏差在整个测量范围内不是恒定的；然后对结果进行反向转换以得出百分比差异。

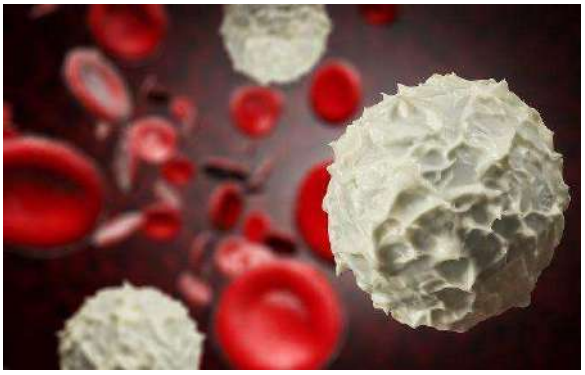
对角线图包含所有数据点。因为末梢血样本 DFA 试纸条测试高于 250 U/L 是不可靠地，Bland-Altman 分析所有平台包括 DFA 试纸条测试，一个值 ≥ 250 U/L 的数据点对被排除在分析之外，以免不适当地扭曲差异分析。用于计算校正因子的数据同样被截断。用于比较实时与远程结果读数的数据没有以这种方式截断。

使用线性回归拟合校正因子（校准方程），通过末梢血品试纸条结果预测血清样品试纸条结果（使用线性回归是因为 DFA 试纸条测量的血清 ALT 浓度被视为连续变量。）

结果

研究背景和参与者

2014 年 2 月至 6 月期间，从 BIDMC 肝脏中心和 BIDMC 传染病诊所招募了 96 名参与者。参与者的中位年龄为 56 岁（范围 22 ~ 79 岁），68%为男性。中位临床 ALT 值（Roche/Hitachi）为 94 U/L（范围 18 ~ 752 U/L）。60 名参与者感染了丙型肝炎病毒，8 名感染了艾滋病毒，2 名感染了乙型肝炎病毒。该人群中其他肝病的病因包括自身免疫性肝炎（n = 2），血色病（n = 2），非酒精性脂肪性肝病（n = 3），α-1 抗胰蛋白酶缺乏症（n = 2），以及继发于药物治疗的急性肝功能衰竭（n = 1）。



研究测试

末梢采血的房间中位温度为 22.7℃（范围 19.5 ~ 25.9℃），中位湿度为 26%（范围 15% ~ 47%）。读取设备房间的中位温度为 23℃（范围 22 ~ 26℃），中位湿度为 21%（范围 15% ~ 47%）。将设备从末梢血采血房间运送到设备被读取的房间之前经过的中位时间为 2 分 30 秒。根据温度/读数时间图将所有装置孵育适当的总时间长度（图 1D）；孵育时间基于阅读室中的温度（方法），中值孵育时间为 25 分钟（范围 22 ~ 25 分钟）。

大多数患者在采完末梢血后立即直接进入临床实验室进行静脉采血。少数患者在采末梢血之前进行静脉穿刺，或者由于临床时间安排的延误，在就诊时进行静脉穿刺（对于这些后来的患者，在采集末梢血后 2 小时内进行静脉穿刺）。对于所有患者，静脉血清标本同一天在 Roche/Hitachi 平台（根据 BIDMC 临床常规）测试。随后使用 DFA 试纸条和 Abaxis Piccolo 自动平台（方法）对血清进行研究测试；在该额外测试之前，将血清储存中位时间 5 天左右（几乎所有样品在 7 天内测试）。

96 名参与者中有 3 名最初入组，但最终未进行静脉血 ALT 测试；两个人忘记完成实验，一个人因为静脉穿刺困难无法完成实验。由于阴性对照区的填充失败，来自三个人的末梢血样本在试纸条测试中产生无效结果（图 1E）。没有观察到由于溶血或阳性对照失败导致的无效测试。最终，91 名患

者提供了有效的末梢血样本和用于 ALT 测试的静脉血清样本（Roche/Hitachi）。丢失的血清可用于这 91 名患者中的 88 名（用于 DFA 试纸条和 Abaxis Piccolo 测试）。未报告任何不良事件。

试纸条和自动化测量 ALT 的性能比较

在我们的临床评估之前，对 Abaxis Piccolo 分析仪和 DFA 试纸条测试进行了内部精确度研究；该评价结果如表 1 所示。数据表明 Abaxis Piccolo 和 DFA 试纸条在性能上相当，并产生可重复的结果。由于试纸条测试是比色法半定量测试，因此预期试纸条测试（与 Abaxis 相比）报告的%CV 略高，而 Abaxis Piccolo 测定是定量测试。

如上所述（方法），对于前 42 名患者，收集第一滴末梢血并应用于 DFA 试纸条测试。因为初步结果已经表明末梢血结果系统地低于对应的血清结果（下图），并且因为一些（但不是全部）末梢血测试方案建议擦除第一滴血液以避免混入过多的组织液，我们调整了接下来 54 名患者的程序，擦去第一滴血液，采集第二滴血液并应用于测试。为了评估末梢血采集过程中的这种变化是否对我们的结果有任何影响，进行了双样本 t 检验以比较 DFA 试纸（末梢血）和 Abaxis Piccolo（血清）测试结果之间的平均差异。两个不同程序比较的 p 值为 0.65，表明平均差异不受样品采集程序变化的影响；因此，将两个程序的结果合并用于分析。

表1. 基于血清标准的DFA纸ALT测试和Abaxis Piccolo ALT测试的精确度

	血清标准1	血清标准2	血清标准3
ALT (U/L) Abaxis Piccolo，mean ± SD (%CV)	61.8 ± 3.77 (6.10)	152.4 ± 2.51 (1.65)	264.8 ± 5.26 (1.99)
ALT (U/L) DFA paper，mean ± SD (%CV)	60 ± 10.54 (17.57)	141 ± 9.94 (7.05)	254 ± 8.43 (3.32)

（DFA，全部测试；ALT，丙氨酸氨基转移酶；SD，标准差；CV，变异系数）

DFA 纸测试末梢血和对应血清样品的 ALT 结果，彼此进行比较，并与在两个自动化平台上测试血清的结果进行比较（图 2 和 3）。图 2 显示了对来自一个人样品的两种不同 ALT 结果的直接比较结果：DFA 纸末梢血结果与 DFA 纸血清结果对比（图 2A），DFA 纸末梢血结果与 Abaxis Piccolo 血清结果对比（图 2B），DFA 纸血清结果与 Abaxis Piccolo 血清结果对比（图 2C），以及 Roche/Hitachi 平台血清结果与 Abaxis Piccolo 血清结果对比（图 2D）。

图 2A 显示，DFA 纸末梢血 ALT 结果系统地低于在 DFA 纸对应血清样品的 ALT 结果，大多数数据点位于相等线之上。类似地，末梢血样品 ALT 结果系统地低于 Abaxis Piccolo 血清样品的结果（图 2B），该自动化平台在优化期间校准 DFA 纸测试。为了确保最佳的末梢血样品体积，我们评估了毛细管的精确度和准确度，以便采集预期的水和血液体积。水样采集的平均体积为 35.6 μL，%CV 为 2.79%（n = 100），血样采集的平均体积为 33.9 μL，%CV 为 2.97%（n = 100），表明体积研究过程中在设备上吸取的末梢血是可靠的。

对于血清样品，来自 DFAs 试纸条测试的 ALT 结果显示与 Abaxis Piccolo 分析仪的结果非常一致（图 2C）。有趣的是，Abaxis Piccolo 的 ALT 结果系统地低于 Roche/Hitachi 平台上测试的相同样品的 ALT 结果（图 2D）。

接下来，采用 Bland-Altman 分析方法[22]（方法；图 3）。如上所述，DFA 试纸条测试末梢血的 ALT 值系统地低于 DFA 试纸条测试血清的值（偏差-23.6 U/L，图 3A，表 2）或 Abaxis 血清的值（偏差-18.4 U/L，图 3B，表 2）。对于血清，试纸条结果和 Abaxis 结果之间存在极好的一致性，偏差可以忽略不计（+4.5 U/L，图 3C，表 2）。血清的 Abaxis 结果平均比 Roche/Hitachi 结果低 8.6%（图 3D，表 2）；值得注意的是，这些平台之间的类似系统差异也已在使用这些平台的实验室进行的熟练度测试中得到证明（www.api-pt.com；见讨论）。仅对 Abaxis 与 Roche/Hitachi 比较需要对数转换（图 3D；参见方法）。为了便于直接比较 DFA 试纸条和 Abaxis 平台血清、Abaxis 平台血清与和 Roche/Hitachi 血清的相对偏差值，我们还计算了 DFA 试纸条血清与 Abaxis 的对数标度平均偏差和返回-转换为给出百分比差异（表 2）。DFA 试纸条测试的血清平均值比 Abaxis 高 2.4%，但这与无偏差无显著差异（95% CI 偏差：-2.4% ~ 7.6%），而 Abaxis 和 Roche/Hatachi 之间存在显著差异（平均偏差-8.6%，95% CI -10.7%～-6.5%）（表 2）

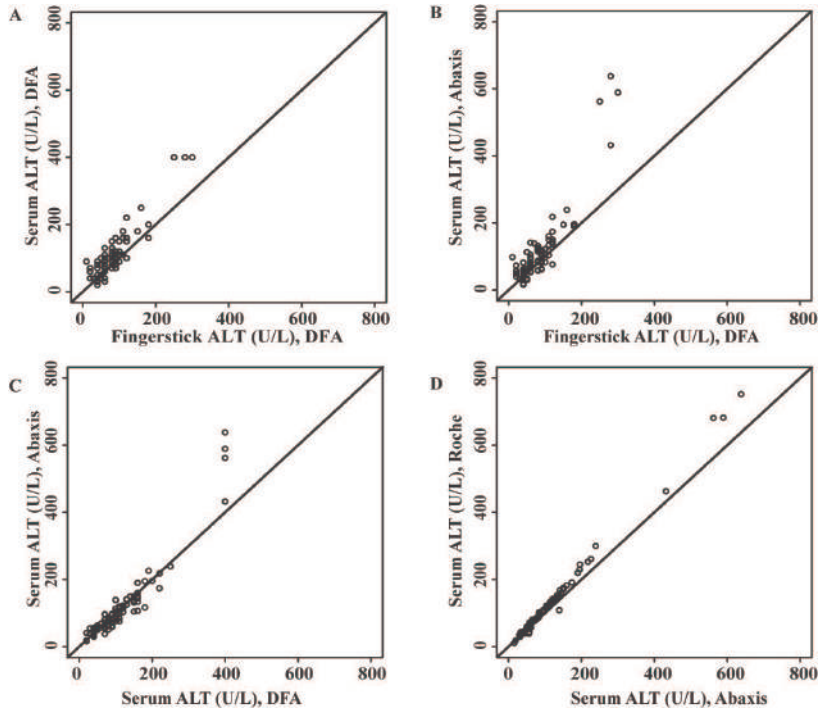


图 2. 末梢血或血清样品不同平台 ALT 结果图。角黑线代表平等线。**A.** 末梢血与对应血清的 DFA 纸测试结果比较。**B.** DFA 纸末梢血结果与 Abaxis Piccolo 测试结果比较。**C.** 血清的 DFA 纸测试结果与血清的 Abaxis Piccolo 测试结果比较。**D.** 血清样品的两个自动平台（Abaxis Piccolo 和 Roche/Hitachi）的结果比较。

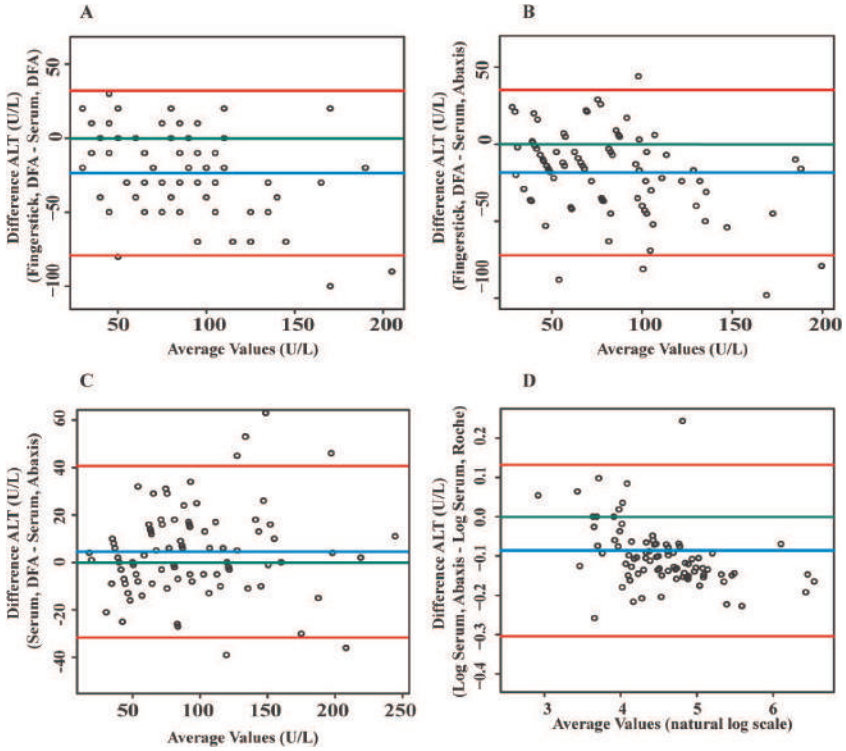


图 3. Bland-Altman 绘图评估不同平台末梢血或血清样本 ALT 结果之间的一致性。每个图中的蓝线代表两种方法之间的“偏差”或平均差异。红线代表 95%限制。 绿线表示无偏差线。**A.** DFA 纸末梢血和血清样本测试结果之间的差异。**B.** 末梢血 DFA 纸测试结果和 Abaxis Piccolo 测试血清结果之间的差异。**C.** 血清 DFA 纸测试结果与血清 Abaxis Piccolo 测试结果之间的差异。**D.** 两个自动平台（Abaxis Piccolo 和 Roche/Hitachi）测试血清样品结果的对数转换之间的差异。

对于血清样品，来自 DFAs 试纸条测试的 ALT 结果显示与 Abaxis Piccolo 分析仪的结果非常一致（图 2C）。有趣的是，Abaxis Piccolo 的 ALT 结果系统地低于 Roche/Hitachi 平台上测试的相同样品的 ALT 结果（图 2D）。

接下来，采用 Bland-Altman 分析方法[22]（方法；图 3）。如上所述，DFA 试纸条测试末梢血的 ALT 值系统地低于 DFA 试纸条测试血清的值（偏差-23.6 U/L，图 3A，表 2）或 Abaxis 血清的值（偏差-18.4 U/L，图 3B，表 2）。对于血清，试纸条结果和 Abaxis 结果之间存在极好的一致性，偏差可以忽略不计（+4.5 U/L，图 3C，表 2）。血清的 Abaxis 结果平均比 Roche/Hitachi 结果低 8.6%（图 3D，表 2）；值得注意的是，这些平台之间的类似系统差异也已在使用这些平台的实验室进行的熟练度测试中得到证明（www.api-pt.com；见讨论）。仅对 Abaxis 与 Roche/Hitachi 比较需要对数转换（图 3D；参见方法）。为了便于直接比较 DFA 试纸条和 Abaxis 平台血清、Abaxis 平台血清与和 Roche/Hitachi 血清的相对偏差值，我们还计算了 DFA 试纸条血清与 Abaxis 的对数标度平均偏差和返回-转换为给出百分比差异（表 2）。DFA 试纸条测试的血清平均值比 Abaxis 高 2.4%，但这与无偏差无显著差异（95% CI 偏差：-2.4% ~ 7.6%），而 Abaxis 和 Roche/Hatachi 之间存在显著差异（平均偏差-8.6%，95% CI -10.7%～-6.5%）（表 2）。

表2. 图3A-3D中显示的Bland-Altman比较的偏差值和相关的95%置信区间（CI）

	平均偏差值，U/L	95%CI	平均偏差值，%	5% CI
A. 末梢血，DFA—血清，DFA	-23.6	-29.7～-17.4	n/a	n/a
B. 末梢血，DFA—血清，Abaxis	-18.4	-24.3～-12.5	n/a	n/a
C. 血清，DFA—血清，Abaxis	4.5	0.6～8.5	2.4	-2.4～7.6
D. Log 血清，Abaxis—Log 血清，Roche	n/a	n/a	-8.6	-10.7～-6.5

n/a，不适用

鉴于末梢血中ALT浓度（[ALT]）与来自同一个体的血清样本之间观察到的系统差异，计算DFA纸测试的校正因子回归方程，以便将来使用以匹配末梢血与血清ALT结果，如下：

$$\text{血清 [ALT]} = 14.81 + 1.12 \times \text{末梢血 [ALT]}$$

回归系数 p 值为 0.03（截距为 14.81）和 < 0.01（试纸条 [ALT]，即 1.12）。我们还通过进行似然比检验来评估包含的二次项是否会对模型的拟合做出显著贡献，但是，由于没有进行测试，我们最终的模型只包含末梢血试纸条 ALT 值的线性项。

远程结果解释

我们将实时读取的设备结果与从不同分辨率手机摄像头捕获设备/读取指南的图像中读取的结果进行比较（2MP vs. 8MP，分别被选为发展中国家和发达国家常用的手机摄像头的代表）和发短信到异地读者（方法）。为了评估一致性，直接绘制数据（图 4A 和 4B），并通过 Bland-Altman 分析评估差异（图 4C 和 4D）。 实时读取和读取 200 万像素拍照手机图像（偏差+5.5 U/L，95% CI 0.1 ~ 10.9）或 800 万像素拍照手机图像（偏差-6.2 U/L，95% CI -12.4 ~ -0.0）（图 4C 和 4D）。

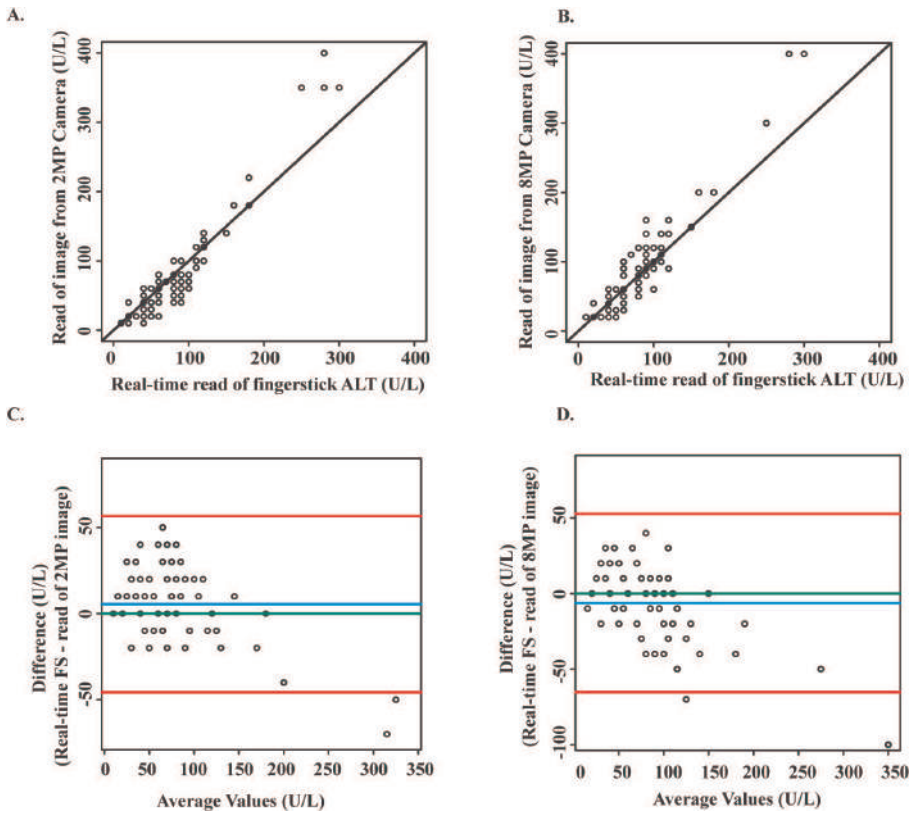


图 4. 远程与实时读取 DFA 试纸条 ALT 结果（针对末梢血样品）。**A.** 实时读取与 2 MP 图像读取的关系图。**B.** 实时读取与 8 MP 图像读取的关系图。对于 A 和 B，对角实线表示相等的线。**C.** Bland-Altman 实时读取和读取 2 MP 图像之间差异的图表。**D.** Bland-Altman 实时读取和 8 MP 图像读取之间差异的图表。对于 C 和 D，蓝线表示通过两种读取方法获得的结果之间的偏差/平均差异。红线代表 95%限制。绿线表示无偏差线。

讨论

我们的研究结果表明，DFA 试纸条 ALT 测试的性能与世界各地临床实验室广泛使用的标准自动化平台的性能相当。我们发现 DFA 试纸条测试对于血清测试是高度准确的，与优化的参考方法（Abaxis）相比，优于两种参考方法（Abaxis 和 Roche/Hitachi）相互匹配。我们在 Abaxis Piccolo 与 Roche/Hitachi 平台测量的血清 ALT 结果之间观察到的系统约 9%差异非常类似于使用这两个平台的临床实验室进行的标准化能力测试所观察到的差异（www.api-pt.com）。这一观察应该提醒临床医生，在没有 ALT 测量的国际标准的情况下，任何给定的自动化平台都不一定能提供“正确的答案”。我们承认血清在 Abaxis 平台上进行测试后在 Roche/Hitachi 平台（通常约 5 天后）进行了测试，但也注意到在 4°C 储存的血清中测得的 ALT 值保持稳定约一周[23]。

重要的是，我们注意到在末梢血与对应血清样本 ALT 值之间的系统差异，末梢血 ALT 值始终低于血清中的 ALT 值。这种差异表明末梢血和静脉血之间 ALT 值的固有差异，正如已经在几种其他分析物中观察到的那样[24-27]。我们的研究结果可能与 DFA 试纸条 ALT 测试有关，因为 FDA 已批准用于测量末梢血 ALT 的 POC 平台（Roche Reflotron[28]和 Alere Cholestech LDX [29,30]）报告没有系统偏差或末梢血和静脉血之间的差异。然而，总结这两项测试可用信息的回顾表明，这些测试实际上并未使用 ALT 值升高患者的末梢血样本进行验证，而是仅使用来自正常至轻度升高的患者末梢血样本（例如最大值为 65 U/L）ALT 值[28-30]。如果测试仅包括 ALT 值正常至轻度升高的患者，我们在末梢血和血清之间观察到的系统差异很容易被遗漏。鉴于没有数据比较 ALT 升高患者的末梢血与血清样本中的 ALT 水平，我们对这两种样本类型基于试纸条测试结果的一致性没有先验预期。据我们所知，这是第一项评估 POC 转氨酶试验对患者人群中末梢血和血清样本的性能研究，该患者具有广泛的基线 ALT 值。不幸的是，我们的研究受限于一个事实，在本研究开始之前，Alere Cholestech LDX 被 FDA 召回，从而阻止了在两个平台上用末梢血样本 ALT 结果之间的直接比较。通过使用不同的样本类型，样本收集方法和比较方法，使这些平台上有限的其他文献的评估变得复杂。在独立的临床试验报告[31]中，Green 等人比较了 Roche-Hitachi 模块化分析仪测量的血清 ALT 值和 Cholestech

LDX 测量的全血 ALT 值，发现 Cholestech 测量的 ALT 值系统低于 Roche-Hitachi 测量值。然而，James 等[32]的另一项研究指出，与使用参考方法测量的血清 ALT 值相比，在 Reflotron 上测量时，全血 ALT 值更高。当使用 Reflotron 分析全血和血浆时，作者观察到 ALT 结果的良好一致性，并且类似地，当通过 Reflotron 和参考方法分析血清时，结果支持他们的结论，即血清和全血值之间存在不一致。

需要进一步研究以明确断定静脉和末梢血液中 ALT 浓度存在固有差异。我们注意到，在用于测试全血的 DFA 试纸条装置上存在覆盖膜，在装置优化期间的先前实验中显示可以减缓蒸发，否则与相同体积的血清中测量的值相比，会降低全血中测量的 ALT 值。因为覆盖膜用于全血的装置完全校正了蒸发对该样品类型的影响，并且从全血和血清获得了均衡的结果，所以在该研究中用于末梢血液（但不是血清）的装置同样具有覆盖膜。对于末梢血，蒸发效果可能没有完全校正，因此我们发现末梢血对比血清样本的 ALT 值较低，但我们发现这不太可能。无论如何，我们在针对血液样本的 DFA 试纸条测试结果之间观察到的系统差异允许我们计算出现在可以应用于末梢血结果的校正因子，以允许它们匹配从试纸条测试获得的血清结果（反过来又是与自动化平台结果密切配合）。因此，通过使用该校正因子来调整读取指南，将使用 DFA 试纸条测试的末梢血试结果校准到 Abaxis Piccolo 平台上的血清测试结果。需要使用新调整的阅读指南进行进一步的临床评估，以便正式验证这个校正因子。

该研究进一步证明了远程设备读取的可行性，其中由简单的手机摄像机捕获的设备和读取指南的图像可以被发送到中心位置并且由受过训练的读取器在那里（在计算机屏幕上）读取。对 2MP 和 800 万像素摄像机图像的读取与设备的实时读取非常一致（图 4）。这种方法可以在不需要熟悉设备结果解释的读者在现场进行测试的情况下进行测试，从而为基于家庭的测试打开了大门。

总之，我们已经证明了 DFA 试纸条张测试与 ALT 测量自动化平台之间的良好一致性，并表明远程读取试纸条设备是可行的。我们的研究结果还揭示了临床 ALT 测量的两个关键方面：a）样本类型（末梢血 vs. 血清）的 ALT 水平的系统差异，和 b）由 FDA 批准的不同自动化平台提供的 ALT 测量的变异性。该 POC 装置具有显著的全球潜力，可用于监测潜在肝毒性药物（包括结核病和艾滋病毒）患者的 DILI。

基于这些发现，我们提出通过内源性抑制剂抑制流入血清的循环 ACE 的活性，该抑制剂提供抑制循环 ACE 活性的进化保守机制（图 10）。

致谢

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作者贡献

构思并设计实验：SJ RR FN EC RS RB MC NA SK NRP
完成实验：SJ RR EC RS SK NRP
分析数据：SJ RR FN EC RS SK NRP
贡献试剂/材料/分析工具：SJ FN EC SK
撰写本文：SJ RR FN EC RS SK NRP
协助患者招募：RB MC NA。

Performance of an Optimized Paper-Based Test for Rapid Visual Measurement of Alanine Aminotransferase (ALT) in Fingerstick and Venipuncture Samples

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Competing Interests: The authors have read the journal's policy and have the following conflicts to

Abstract

Background

A paper-based, multiplexed, microfluidic assay has been developed to visually measure alanine aminotransferase (ALT) in a fingerstick sample, generating rapid, semi-quantitative results. Prior studies indicated a need for improved accuracy; the device was subsequently optimized using an FDA-approved automated platform (Abaxis Piccolo Xpress) as a comparator. Here, we evaluated the performance of the optimized paper test for measurement of ALT in fingerstick blood and serum, as compared to Abaxis and Roche/Hitachi platforms. To evaluate feasibility of remote results interpretation, we also compared reading cell phone camera images of completed tests to reading the device in real time.

Methods

96 ambulatory patients with varied baseline ALT concentration underwent fingerstick testing using the paper device; cell phone images of completed devices were taken and texted to a blinded off-site reader. Venipuncture serum was obtained from 93/96 participants for routine clinical testing (Roche/Hitachi); subsequently, 88/93 serum samples were captured and applied to paper and Abaxis platforms. Paper test and reference standard results were compared by Bland-Altman analysis.

Findings

For serum, there was excellent agreement between paper test and Abaxis results, with negligible bias (+4.5 U/L). Abaxis results were systematically 8.6% lower than Roche/Hitachi

declare: Patent application filed pertaining to device: U.S. Provisional Patent Application No. 61/555,977 "Quantitative microfluidic devices" (Kumar S. and Jain S. are listed as inventors of this application). This does not alter their adherence to all the PLOS ONE policies on sharing data and materials. There are no other relevant declarations relating to employment, consultancy, patents, products in development or modified products, etc.

results. ALT values in fingerstick samples tested on paper were systematically lower than values in paired serum tested on paper (bias -23.6 U/L) or Abaxis (bias -18.4 U/L); a correction factor was developed for the paper device to match fingerstick blood to serum. Visual reads of cell phone images closely matched reads made in real time (bias +5.5 U/L).

Conclusions

The paper ALT test is highly accurate for serum testing, matching the reference method against which it was optimized better than the reference methods matched each other. A systematic difference exists between ALT values in fingerstick and paired serum samples, and can be addressed by application of a correction factor to fingerstick values. Remote reading of this device is feasible.

Introduction

Point-of-care (POC) diagnostics are particularly desirable for diagnosis and medical management in resource-constrained settings. In such settings, the centralized laboratory testing upon which clinicians in resource-rich settings rely may be unavailable or cost-prohibitive. If centralized testing is available, lengthy results turn-around time can lead to patients becoming lost to follow-up, adversely impacting outcomes. With these barriers in mind, there has been a recent explosion in the development of POC diagnostics for many applications [1–3].

One example of a defined need for POC testing is for monitoring of transaminases for diagnosis and management of drug-induced liver injury (DILI). Transaminase testing is particularly necessary for persons on medications for treatment of HIV and TB, as many of these medications are known to be hepatotoxic [4,5]. Transaminase monitoring is also valuable to evaluate those with underlying liver diseases such as hepatitis B or hepatitis C. Collectively, these diseases disproportionately affect those in resource-limited settings, and with limited or no access to transaminase testing in many of these settings, patients are put at increased risk of complications of DILI. Typically, transaminase monitoring requires equipment for a venous blood draw, a trained phlebotomist, centrifugation to separate serum or plasma, and testing on a large automated platform. Such platforms are expensive and require highly trained technicians for testing and maintenance, making them impractical for use and scale-up in many developing countries. Because of these obstacles, in many resource-limited settings, patients on potentially hepatotoxic medications receive minimal or no monitoring during treatment. Automated platforms for POC transaminase testing have been developed (Roche Reflotron and Alere Cholestech); however, the Choletech ALT test is currently off the market worldwide and the Reflotron is currently off the market in the US.

We have recently developed a paper-based, multiplexed, microfluidic assay to visually and semi-quantitatively measure alanine aminotransferase (ALT) in a fingerstick sample [6–8]. This device is based on "patterned-paper" technology, in which a wax-based printer is used to create a series of hydrophobic barriers and hydrophilic channels that guide fluid wicking through the paper both laterally and vertically. As fluid flows directionally through the layers of patterned paper, it contacts zone-specific assay reagents, allowing multiple reactions to be performed in parallel on a single sample. Our assay has been designed to yield a rapid (~18–30'), semi-quantitative, visual result; moreover, it is portable, disposable, requires no power, and is anticipated to cost ~$0.10/test, making it ideal for resource-limited settings. Similar paper-based microfluidic technology is being evaluated for a wide range of applications,

including (as examples) glucose and protein measurement, detection of bacteria, detection of hepatitis C antibody, detection of cancer biomarkers, and blood typing [9–15]. To our knowledge, within this emerging class of paper-based microfluidic platforms, our paper-based ALT test is currently the closest to actual clinical application.

Early pre-clinical testing on clinical serum and whole blood specimens demonstrated that the paper-based device could yield visual measurements with >90% accuracy [6] and was therefore ready for field testing. Thereafter, the first fingerstick evaluation of the test was performed in 600 HIV-infected persons receiving care in a busy HIV clinic in Vietnam [7], considered an ideal target setting for application of this test. That evaluation study demonstrated that the device operation and reading process were both feasible and extremely reproducible in this target setting, but highlighted the need for further device optimization to reduce hemolysis rates and improve accuracy. The device subsequently underwent extensive optimization, including sourcing of a new plasma separation membrane, treatment of this membrane with an anti-hemolytic coating to reduce hemolysis rates, reformulation of assay chemistry, and recalibration against an automated reference standard, Abaxis Piccolo [16].

Here, we present results of a validation study of the optimized ALT test as performed in ambulatory outpatients with liver disease or on hepatotoxic medications, each of whom required ALT monitoring as part of routine care. The goals of this study were to evaluate the performance of the optimized paper-based test for measurement of ALT levels in both fingerstick blood and venipuncture serum for patients with a range of baseline ALT levels, as compared to results of testing the serum on two FDA-approved automated platforms in wide clinical use. As a result, we were able to evaluate the impact of sample type on results of the paper test, as well as to compare performance of the paper test to that of each automated platform. We also assessed the potential utility of cell-phone cameras to allow remote interpretation of the paper test results.

Materials and Methods

Device Design, Production and Storage

The "patterned paper" ALT tests are created using wax-based printing technology; after printing, paper layers are heated to 110°C, which melts the wax and allows it to permeate through the thickness of the paper (GE Whatman Chromatography Grade 1, Piscataway, NJ), creating microfluidic, hydrophilic detection zones surrounded by hydrophobic wax barriers [17–20]. The ALT test is constructed by stacking two such patterned paper layers, along with a plasma separation membrane disc (Primecare NX-membrane, International Point of Care Inc. Toronto, CA) and lamination films, to create a 3D device (Fig 1A). A cover film (2MIL low density polyethylene, Warp Bros, Chicago IL) providing protection against sample evaporation is added to devices to be used with fingerstick whole blood samples; this cover film is not applied to devices to be tested using serum. The layers are adhered together using patterned pressure-sensitive adhesive films (Flexcon Inc, Spencer, MA). The plasma separation membrane separates red and white blood cells from plasma within fingerstick whole blood, allowing plasma to wick to the detection zones. The plasma separation membrane is treated with an anti-hemolytic coating to prevent hemolysis of cells during filtration.

The ALT test utilizes a peroxidase-based colorimetric assay [6], producing a red color in the presence of elevated ALT. The intensity of the red color of the test spot is directly proportional to the concentration of ALT in the sample, allowing semi-quantitative, visual interpretation of the results by comparison with a reference color chart or "read guide" (Fig 1B). The read guide also allows the user to place the ALT result in one of three clinically relevant bins [<3X upper limit of normal (ULN), 3-5X ULN, and >5X ULN] used in the management of patients at risk

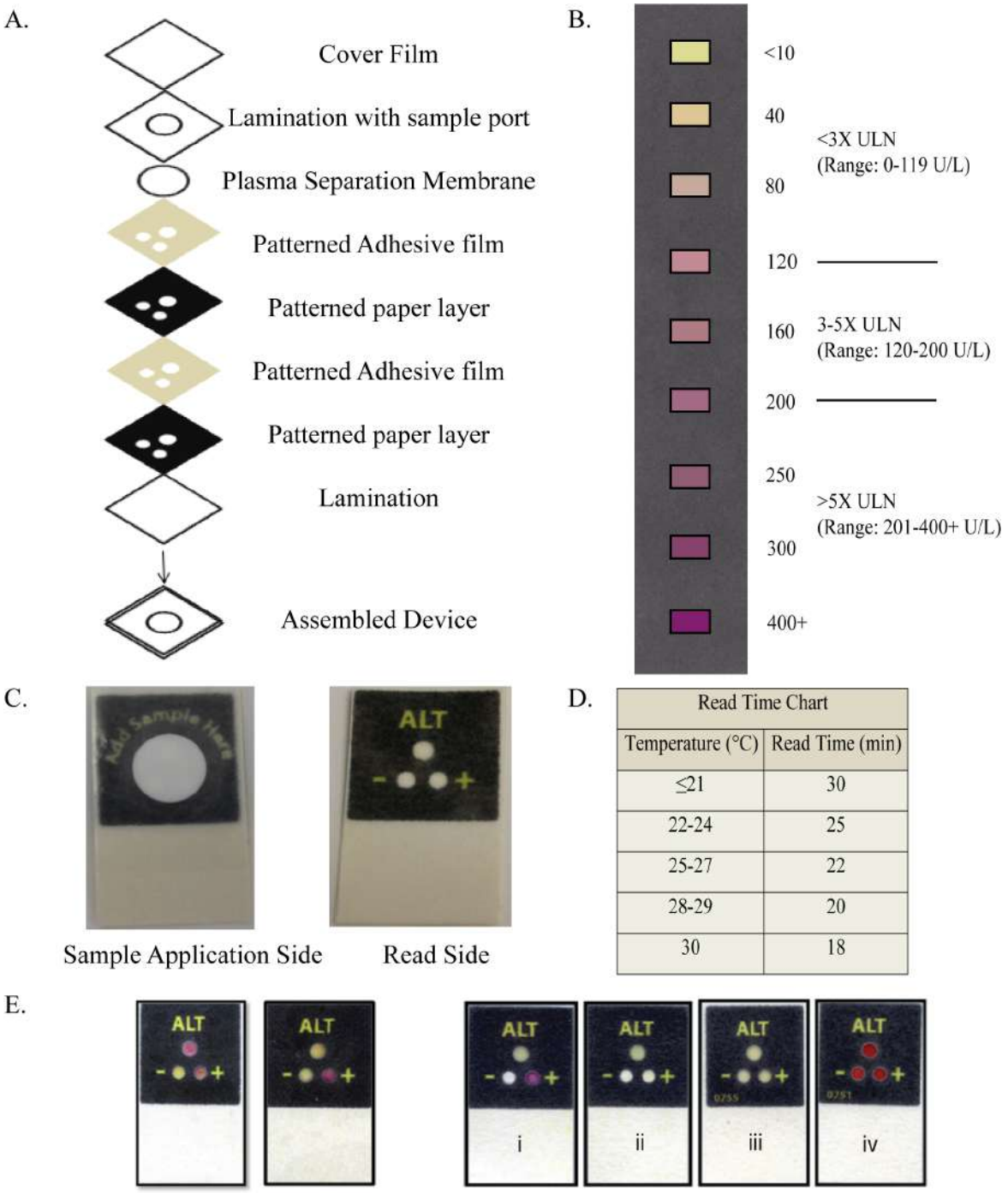

Fig 1. Schematic of the 3-Zone DFA paper-based alanine aminotransferase (ALT) test. A. The DFA ALT test is constructed by assembling two patterned paper layers, a plasma separation membrane disc, and protective lamination films to create a 3-dimensional device. A cover film is applied to devices to be used with fingerstick whole blood samples; this cover film is not applied to devices to be used with serum samples. All layers are adhered together using patterned, pressure-sensitive adhesive films. B. The test utilizes a peroxidase-based colorimetric assay to provide a semi-quantitative ALT result determined through visual comparison with a reference color chart. The color chart also allows the user to place the ALT result in one of three categorical bins: <3X upper limit of normal (ULN), 3-5X ULN, and >5X ULN. C. A fingerstick blood sample is applied to the sample port on the "sample application side" of the test. Blood cells are separated by the plasma separation membrane, allowing plasma to wick into the device and react with reagents dried onto individual detection zones; results are viewed on the "read side." D. The results are read after an incubation time that corresponds to the ambient

temperature. E. Two control zones on the test are used to determine the validity of the test results. Examples of valid devices are shown in the two images on the left. Four invalid examples are shown on the right, as follows: i. Insufficient sample volume, evident by the lack of a yellow color in the negative control zone. ii. Insufficient sample volume (as in i) and positive control failure, latter indicating inactive reagents at the time of testing. iii. Positive control failure. iv. Hemolyzed sample, indicated by the presence of a red color in the negative control zone.

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for DILI. The negative control spot serves as an indicator for sample adequacy (adequate sample volume, no hemolysis), and the positive control spot serves as an indicator for reagent activity at the time of testing (Fig 1C and 1E). In this assay, as in any other enzymatic reaction, the rate of the reaction is temperature-dependent. Therefore, the time window within which the test must be read is determined using a temperature/read-time chart (Fig 1D). Each control zone is interpreted as either 'VALID' or 'INVALID'. An 'INVALID' on either control zone invalidates the device [7] (Fig 1E).

400 devices were fabricated in parallel at Diagnostics For All (DFA) using established techniques [6,7]. The paper test measures ALT via its enzymatic activity in a three-step reaction. The test zone contains L-alanine and α-ketoglutarate in the first layer (as substrates) and ALT, present in the sample, catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate producing pyruvate and glutamate. In the second step, pyruvate oxidase, contained in the second layer of the ALT test zone, oxidizes the pyruvate to acetylphosphate and hydrogen peroxide. In the final step of the reaction, horseradish peroxidase (HRP) catalyzes the reaction of the hydrogen peroxide with 4-aminoantipyrine and m-tolyldiethanolamine (TDA) to produce a red/pink colored dye complex that correlates to the ALT concentration in the sample. This enzymatic reaction is similar to that used in other ALT tests, specifically Cholestech LDX (Alere, Inc.), and Reflotron System (Boehringer Mannheim Corp.).

The chemical reactions are as follows:

$$\mathrm{L-alanine} + \alpha\mathrm{-ketoglutarate} \xrightarrow{\text{Alanine Aminotransferase}} \mathrm{pyruvate} + \mathrm{glutamate}$$

$$\mathrm{pyruvate} + \mathrm{phosphate} + \mathrm{O_2} + \mathrm{H_2O} \xrightarrow[\text{TPP, Mg2+}]{\text{Pyruvate Oxidase}} \mathrm{acetylphosphate} + \mathrm{CO_2} + \mathrm{H_2O_2}$$

$$\mathrm{H_2O_2} + 4\mathrm{-aminoantipyrine} + \mathrm{m-tolyldiethanolamine} \xrightarrow{\text{HRP}} \mathrm{Red/Pink\ Dye\ Complex}$$

This formulation produces a stronger color change and larger linear dynamic range when compared to the formulation relying on 4-aminoantipyrine and 3,5-diaminobenzoic acid (DABA) used in previous versions of the DFA paper ALT test [6–8].

In the optimized paper test, spotting of alanine and α-ketoglutarate in a separate layer from the remaining reagents improved stability and performance of the paper test. A stabilizer (bovine serum albumin) was added to all reagents to extend shelf life. Accelerated stability studies showed that the device is stable for approximately 18 months when stored at the recommended temperature range (18–30°C).

The devices were pouched individually in foil-lined aluminum pouches, each with a pillow pack of desiccant, and stored at room temperature for the duration of the study. Devices were quality-tested prior to release for use in this study, per DFA's standard operating procedures.

Within-run precision study

Three standards were prepared by spiking normal human serum (Valley Biomedical Inc, Winchester, VA) with purified ALT (LeeBio Solutions, St. Louis, MO) at target concentrations

(low, medium and high). These samples were blinded to the operator running the precision studies. The three standards were each tested in five replicates on the Hepatic Function Panel, Abaxis Piccolo Xpress (Abaxis, Union City, CA)) [16] and in 10 replicates on the DFA paper tests on the same day. The % coefficient of variation (CV) was calculated for both Abaxis Piccolo and DFA paper tests. This precision study was performed on one lot each of Abaxis Piccolo Hepatic Function Panels and DFA paper devices.

Capillary tube dispensation volume accuracy

A 35μL Microsafe capillary tube (Safe-Tec Inc, Ivyland, PA) was used to collect and dispense fingerstick samples in the clinical study. The dispense volume accuracy of the capillary tube was tested using freshly collected whole blood (Research Blood Components LLC, Brighton, MA), and de-ionized water. The weight of the dispensed volume was measured using a calibrated, analytical balance (NewClassic MF, Mettler Toledo, Columbus OH). %CV was calculated for both de-ionized water and whole blood.

Ethics Statement

This study was approved by the Beth Israel Deaconess Medical Center (BIDMC) Institutional Review Board. The IRB approved use of verbal (rather than written) consent for this minimal risk study; a verbal consent script describing study purpose and procedures was read to each individual, verbal consent was obtained and documented, and the subject was given a study information sheet to keep.

Participants

Participants were recruited from the BIDMC Liver Center and Infectious Diseases outpatient clinics. To be considered for study participation, participants had to be receiving ALT testing that day per clinical routine (collection of venipuncture blood, followed by testing on Roche/Hitachi platform). Recruitment was based on previous ALT results, in order to include patients with ALT values throughout the clinical range; patients with prior ALT $\geq$120 U/L were prioritized for recruitment when possible. Participants were sampled consecutively based on the above selection criteria, and verbal informed consent was obtained. Data on each participant were collected via chart review, and included basic demographic information, results of recent laboratory testing (including ALT levels on the day of enrollment) and primary cause of liver disease if known.

Testing procedure

Fingersticks were performed on study participants by operator 1 using a Surgilance SLN300 lancet (Medipurpose, Duluth, GA). For the first 42 patients, the first drop of fingerstick blood was collected [using a 35μL Microsafe capillary tube (Safe-Tec Inc, Ivyland PA)] and dispensed on the device. For the next 54 patients, the first drop of blood was wiped away and the 35μL sample was collected from the second drop of blood using the capillary tube and dispensed on the device. A count up timer was started after the application of sample. After the fingerstick, patients were sent to the phlebotomy laboratory for venipuncture for routine clinical ALT testing. Operator 1 performing the fingerstick recorded the degree of difficulty (if any) in obtaining the required sample volume and the temperature/relative humidity of the fingerstick room on a study form specific to the study ID number. The device was placed in a petri dish and brought (along with timer and study form) to the incubation room where a second operator (blinded to any prior patient ALT values) interpreted and recorded the results. Operator 2 selected a read

time based on the temperature of the incubation room. Results were reported in U/L (rounded to the nearest 10 U/L). Operator 2 also noted the validity of the device by visually inspecting the control zones for hemolysis, incomplete filling, and reagent function. Incubation room temperature and relative humidity were also recorded.

Following reading of device results, operator 2 photographed the device and read guide together to allow remote reading of the test images. The photographs were taken using two cell phones, one with an 8 mega pixel camera (Samsung Galaxy SII) and another with a 2 mega pixel camera (AT&T Z431). Both sets of camera phone images were texted to two separate email addresses. Two separate operators (operators 3 and 4) read the two image sets using the read guide captured in the images (operator 3 read images from the 2 mega pixel camera and operator 4 read images from the 8 mega pixel camera) on standard laptop monitors. Both operators 3 and 4 were blinded to the results noted by operator 2 in real time.

ALT testing of venipuncture blood was performed by the BIDMC clinical laboratory (serum, Roche/Hitachi platform, without pyridoxal phosphate activation [21]) as per clinical routine. The clinical serum sample was held at 4°C for 5 days and then de-identified and provided to DFA for testing on the DFA paper device and the Abaxis Piccolo analyzer [16]. Testing on the DFA paper devices was performed by another trained reader blinded to both the Piccolo results and to all other results collected in the study.

Statistical Analysis

Agreement between methods of measurements was assessed graphically and through the estimation of the mean difference, or bias, and 95% limits of agreement. Graphs included "diagonal plots" (where one method was simply plotted against the other and evaluated relative to the line of equality) and Bland-Altman plots [22]. For Bland-Altman analysis, logarithmic transformation was used for comparisons in which the mean and standard deviation of the differences were not constant throughout the range of measurement; results were then back-transformed to give percentage differences.

For diagonal plots, all data points were included. Because the DFA paper test is not reliably linear above 250 U/L for fingerstick blood samples, for all Bland-Altman analyses for platform comparisons which included the DFA paper test, pairs of data points in which one of the values was >250 U/L were excluded from analysis, so as not to inappropriately distort the analysis of difference. Data for calculation of the correction factor was similarly truncated. Data for comparison of real-time vs remote results reading was not truncated in this manner.

A correction factor (calibration equation) was fitted using linear regression to predict the paper test result for a serum sample from a paper test result for a fingerstick sample (linear regression was used because the outcome, serum ALT concentration as measured by the DFA paper test, was treated as a continuous variable.)

Results

Study Setting and Participants

96 participants were recruited from the BIDMC Liver Center and the BIDMC Infectious Diseases clinic between February and June 2014. The median age of participants was 56 (range 22 to 79), and 68% were male. The median clinical ALT value (Roche/Hitachi) was 94 U/L (range 18 to 752 U/L). Sixty participants had hepatitis C virus infection, 8 had HIV infection, and 2 had hepatitis B virus infection. Other etiologies of liver disease in this population included autoimmune hepatitis (n = 2), hemochromatosis (n = 2), non-alcoholic fatty liver disease (n = 3), alpha-1 antitrypsin deficiency (n = 2), and acute liver failure secondary to medication (n = 1).

Study testing

The median temperature in the room where the fingerstick was performed was 22.7°C (range 19.5°C to 25.9°C), with median humidity of 26% (range 15% to 47%). Median temperature in the room where the device was read was 23°C (range 22°C to 26°C), with median humidity of 21% (range 15–47%). The median time that elapsed before devices were transported from the room where the fingerstick was performed to the room where the device was read was 2 minutes and 30 seconds. All devices were incubated for the appropriate total length of time per the temperature/read-time chart (Fig 1D); incubation time was based on temperature in the reading room (Methods), and median incubation time was 25 min (range 22 to 25 min).

Most patients proceeded directly to the clinical laboratory for venipuncture immediately after providing a fingerstick sample. A small number had their venipuncture done immediately prior to fingerstick, or while waiting for their clinic visit due to delays in clinic schedules (for these latter patients, venipuncture was performed within 2 hours of fingerstick). For all patients, the venipuncture sample (serum) was tested on the Roche/Hitachi platform (per BIDMC clinical routine) on the same day that it was drawn. The serum was subsequently captured for research testing with both the DFA paper test and the Abaxis Piccolo automated platform (Methods); the serum was stored for a median time of 5 days (almost all samples were tested within 7 days) prior to this additional testing.

Three out of the 96 participants were initially enrolled but ultimately did not have ALT testing performed on venipuncture blood; two forgot to get labs done, and one was unable to get labs done due to difficulty with venipuncture. Fingerstick samples from three individuals generated invalid results on the paper test due to filling failure in the negative control zone (Fig 1E). No invalid tests due to hemolysis or positive control failure were observed. Ultimately, 91 patients provided both a valid fingerstick sample and a venipuncture serum specimen for ALT testing (Roche/Hitachi). Discarded serum was available for capture on 88 of these 91 patients (for testing on the DFA paper test and the Abaxis Piccolo). No adverse events were reported.

Comparative performance of paper and automated tests for measurement of ALT

Prior to our clinical evaluation, a within-run precision study was performed on both the Abaxis Piccolo analyzer and the DFA paper test; results of this evaluation are shown in Table 1. The data indicate that the Abaxis Piccolo and the DFA paper test are comparable in performance and generate reproducible results. The slightly higher %CVs reported for the paper test (vs Abaxis) are expected as the paper test is a colorimetric, semi-quantitative test, whereas the Abaxis Piccolo assay is a quantitative test. \

As noted (Methods), for the first 42 patients, the first drop of fingerstick blood was collected and applied to the DFA paper test. Because initial results already suggested that fingerstick results were systematically lower than paired serum results (below) and because some (but not all) fingerstick testing protocols suggest wiping away the first drop of blood to avoid collection of excess tissue fluid, we adjusted our procedure for the next 54 patients so that the first drop of

Table 1. Precision of the DFA paper-based ALT test and the Abaxis Piccolo ALT test, as performed on serum standards.

	Serum Standard 1	Serum Standard 2	Serum Standard 3
ALT (U/L) Abaxis Piccolo, mean ± SD (%CV)	61.8 ± 3.77 (6.10)	152.4 ± 2.51 (1.65)	264.8 ± 5.26 (1.99)
ALT (U/L) DFA paper, mean ± SD (%CV)	60 ± 10.54 (17.57)	141 ± 9.94 (7.05)	254 ± 8.43 (3.32)

(DFA, Diagnostics For All; ALT, alanine aminotransferase; SD, standard deviation; CV, coefficient of variation)

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blood was wiped away and the second of drop of blood was collected and applied to the test. To evaluate whether this change in fingerstick procedure had any impact on our results, a two-sample t-test was performed to compare the average differences between DFA paper (fingerstick) and Abaxis Piccolo (serum) test results in the groups undergoing the two different procedures. The p-value for that comparison was 0.65, indicating that the average difference was unaffected by the change in the sample collection procedure; therefore, results from the two procedures were pooled for analysis.

ALT results from testing fingerstick and paired serum samples on the DFA paper test were compared to each other and to results from testing serum on the two automated platforms (Figs 2 and 3). The plots in Fig 2 show results for direct comparison of ALT results from two different tests performed on samples from one individual: fingerstick blood on the DFA paper test vs serum on the DFA paper test (Fig 2A), fingerstick blood on the DFA paper test vs serum on the Abaxis Piccolo (Fig 2B), serum on the DFA paper test vs serum on the Abaxis Piccolo (Fig 2C), and serum on the Abaxis Piccolo vs serum on the Roche/Hitachi platform (Fig 2D). Fig 2A shows that ALT results for fingerstick blood tested on the DFA paper test were systematically lower than ALT results for paired serum samples tested on the DFA paper test, as the majority of the data points lie above the line of equality. Similarly, ALT results for fingerstick samples were systematically lower than results for paired serum samples tested on the Abaxis

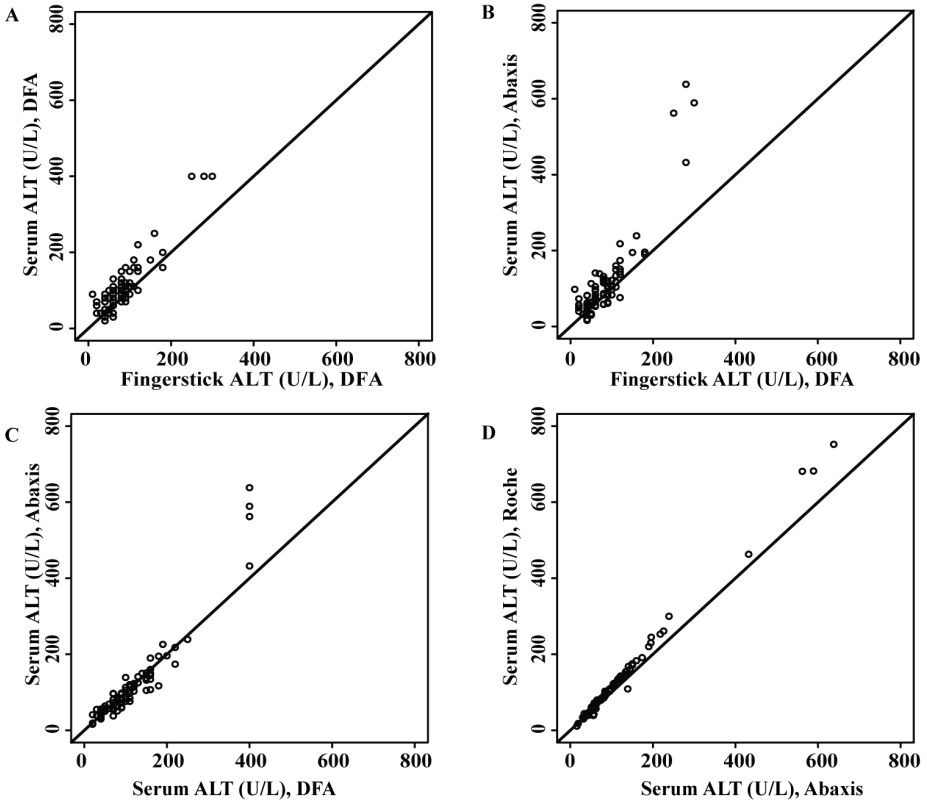

Fig 2. Plots of ALT results generated by different platforms for fingerstick or serum samples. The diagonal black line represents the line of equality. A. Comparison of DFA paper test results for fingerstick blood vs paired serum. B. Comparison of DFA paper test results for fingerstick blood to Abaxis Piccolo test results for paired serum. C. Comparison of DFA paper test results for serum to Abaxis Piccolo test results for serum. D. Comparison of the results of two automated platforms (Abaxis Piccolo and Roche/Hitachi) for serum samples.

doi:10.1371/journal.pone.0128118.g002

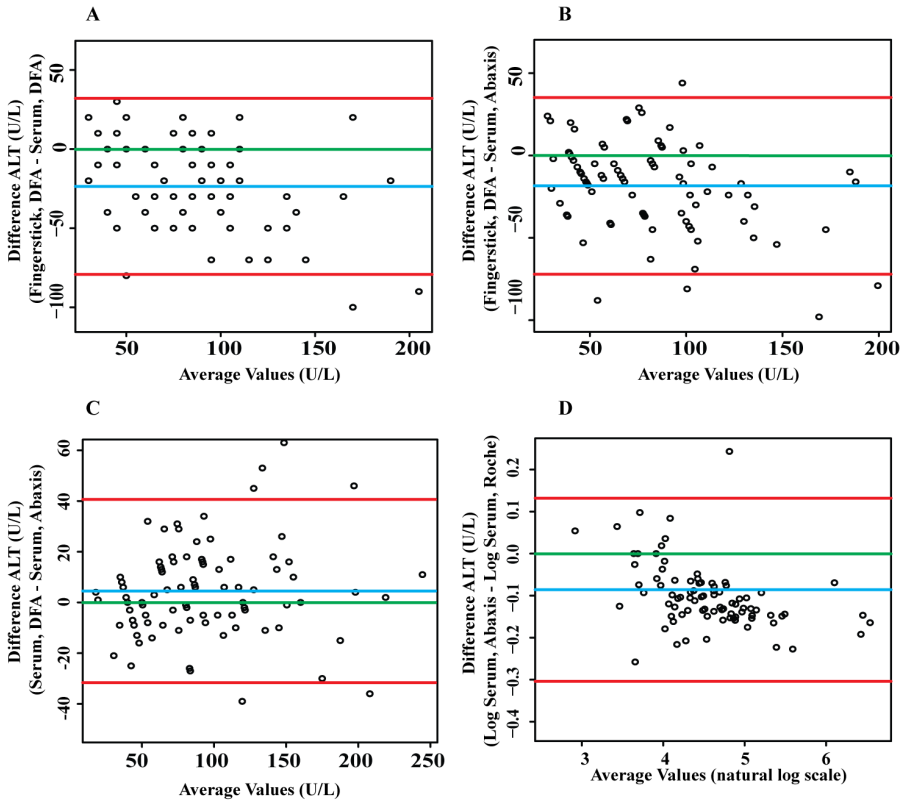

Fig 3. Bland-Altman plots to evaluate agreement between ALT results generated by different platforms for fingerstick or serum samples. The blue line in each plot represents the "bias" or average difference between the two methods. The red lines represent the 95% limits of agreement. The green line represents the line of no bias. A. Difference between DFA paper test results for fingerstick blood vs paired serum. B. Difference between DFA paper test results for fingerstick blood and Abaxis Piccolo test results for paired serum. C. Difference between DFA paper test results for serum and Abaxis Piccolo test results for serum. D. Difference between the log transformation of the results of the two automated platforms (Abaxis Piccolo and Roche/Hitachi) for serum samples.

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Piccolo (Fig 2B), the automated platform against which the DFA paper test was calibrated during optimization. In order to ensure that the optimal fingerstick sample volume was being reproducibly delivered to the test by the plastic capillary tube used for sample collection (Methods), we evaluated the precision and accuracy of the capillary tube for dispensation of expected volumes of water and blood (Methods). The mean volume dispensed for water samples was 35.6 μL with a %CV of 2.79% (n = 100), and the mean volume dispensed for blood samples was 33.9 μL with a %CV of 2.97% (n = 100), indicating that volumes of fingerstick blood dispensed on the devices during the study were reliable.

For serum samples, ALT results from the DFA paper test showed excellent agreement with results from the Abaxis Piccolo analyzer (Fig 2C). Interestingly, ALT results from the Abaxis Piccolo were systematically lower than ALT results from the same sample tested on the Roche/Hitachi platform (Fig 2D).

Next, we evaluated agreement (for the same comparisons) by Bland-Altman analysis [22] (Methods; Fig 3). As noted, ALT values in fingerstick samples tested on the DFA paper test were systematically lower than values in paired serum tested on the DFA paper test (bias -23.6 U/L, Fig 3A, Table 2) or Abaxis (bias -18.4 U/L, Fig 3B, Table 2). For serum, there was excellent

Table 2. Bias values and associated 95% confidence intervals (CI) for Bland-Altman comparisons shown in Fig 3A–3D.

	Mean bias value, U/L	95% CI	Mean bias value, %	95% CI
A. Fingerstick, DFA—Serum, DFA	-23.6	-29.7 to -17.4	n/a	n/a
B. Fingerstick, DFA—Serum, Abaxis	-18.4	-24.3 to -12.5	n/a	n/a
C. Serum, DFA—Serum, Abaxis	4.5	0.6 to 8.5	2.4	-2.4 to 7.6
D. Log Serum, Abaxis—Log Serum, Roche	n/a	n/a	-8.6	-10.7 to -6.5

n/a, not applicable.

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agreement between paper test and Abaxis results, with negligible bias (+4.5 U/L, Fig 3C, Table 2). Abaxis results for serum were 8.6% lower than Roche/Hitachi results on average (Fig 3D, Table 2); notably, a similar systematic difference between these platforms has also been demonstrated in proficiency testing performed by labs using these platforms (www.api-pt.com; see discussion). Logarithmic transformation was only required for the Abaxis versus Roche/Hitachi comparison (Fig 3D; see Methods). To facilitate a direct comparison of the relative bias values for serum on the DFA paper test versus Abaxis and serum on the Abaxis versus Roche/Hitachi, we also calculated the mean bias on the logarithmic scale for serum on the DFA paper test versus Abaxis and back-transformed to give percentage differences (Table 2). Serum on the DFA paper test was 2.4% higher on average than Abaxis, but this was not significantly different from no bias (95% CI for bias: -2.4% to 7.6%), while there was a significant difference between Abaxis and Roche/Hitachi (mean bias -8.6%, 95% CI -10.7% to -6.5%) (Table 2).

Given the systematic difference observed between ALT concentration ([ALT]) in fingerstick vs serum samples from the same individual, a correction factor regression equation was calculated for the DFA paper test for future use to match fingerstick blood to serum ALT results, as follows:

$$Serum\ paper\ [ALT] = 14.81 + 1.12 * Fingerstick\ paper\ [ALT]$$

P-values for the regression coefficients were 0.03 (for the intercept, i.e. 14.81) and <0.01 (for the fingerstick paper [ALT], i.e. 1.12). We also assessed whether inclusion of a quadratic term would contribute significantly to the fit of the model by conducting a likelihood ratio test, but, as it did not, our final model contains only a linear term for the fingerstick on paper ALT value.

Remote results interpretation

We compared device results as read in real time to results read from images of resulted devices/read guides captured by cell phone cameras of different resolution (2MP vs 8MP, selected as representative of cell phone cameras commonly used in the developing and the developed world, respectively) and texted to an off-site reader (Methods). To evaluate agreement, data were plotted directly (Fig 4A and 4B) and differences were evaluated by Bland-Altman analysis (Fig 4C and 4D). There was excellent agreement between the read made in real time and the read of either the 2MP camera phone image (bias +5.5 U/L, 95% CI 0.1 to 10.9) or the 8MP camera phone image (bias -6.2 U/L, 95% CI -12.4 to -0.0) (Fig 4C and 4D).

Discussion

The results of our study indicate that the performance of the DFA paper ALT test is comparable to that of standard automated platforms widely used in clinical labs around the world. We found that the DFA paper test was highly accurate for serum testing, matching the reference

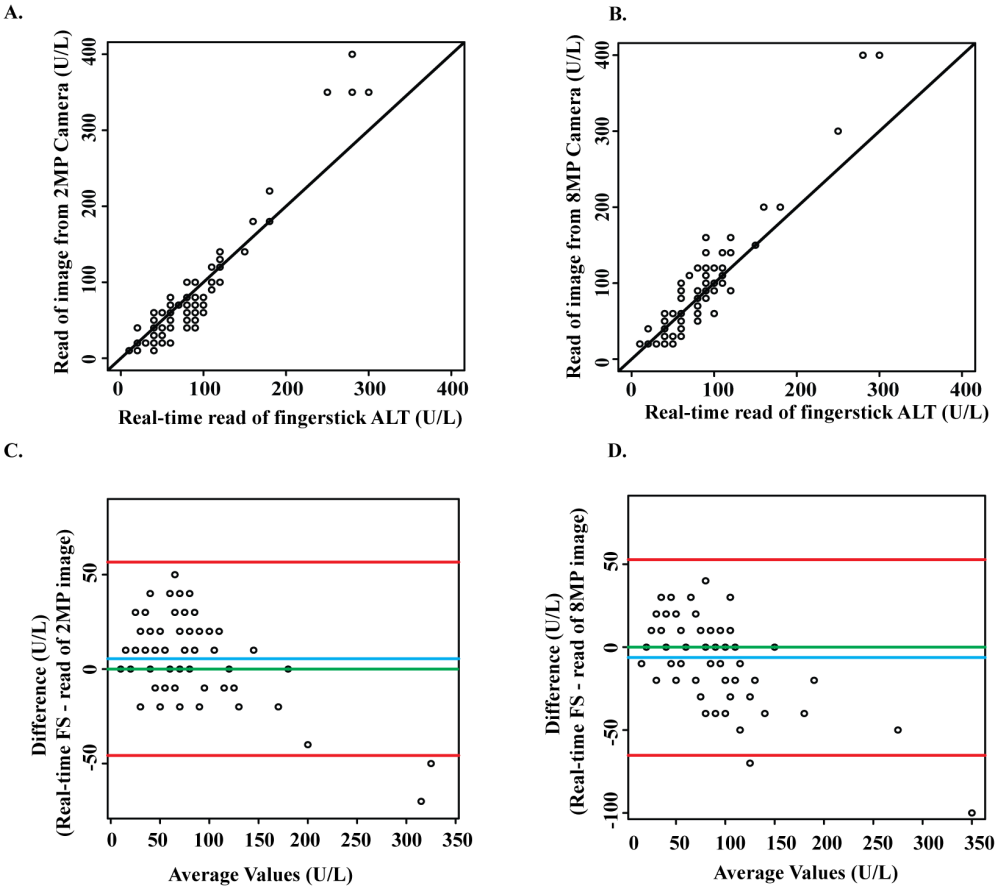

Fig 4. Remote vs real-time reading of DFA paper ALT test results (for fingerstick samples). A. Plot of real-time reads vs reads of 2 MP images. B. Plot of real-time reads vs reads of 8 MP images. For both A and B, the diagonal solid line represents the line of equality. C. Bland-Altman plot of differences between real-time reads and reads of 2 MP images. D. Bland-Altman plot of differences between real-time reads and reads of 8 MP images. For both C and D, the blue line represents the bias/average difference between results obtained by the two reading methods. The red lines represent the 95% limits of agreement. The green line represents the line of no bias.

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method against which it was optimized (Abaxis) better than the two reference methods (Abaxis and Roche/Hitachi) matched each other. The systematic ~9% difference we observed between serum ALT results measured by the Abaxis Piccolo vs Roche/Hitachi platforms is very similar to the difference observed on standardized proficiency testing performed by working clinical laboratories utilizing these two platforms (www.api-pt.com). This observation should serve as a reminder to clinicians that, in the absence of international standards for ALT measurement, any given automated platform does not necessarily provide the "right answer." We acknowledge that serum was tested on the Abaxis platform after it was tested on the Roche/ Hitachi platform (typically ~5 days later), but also note that ALT values measured in serum stored at 4°C remain stable for approximately one week [23].

Importantly, we noted a systematic difference between ALT values measured in fingerstick vs paired serum samples, with measured ALT values in fingerstick blood consistently below measured ALT values in paired serum. This difference suggests an inherent difference in ALT values between capillary and venous blood, as has been observed for several other analytes, e.g. [24–27]. Our findings may be specific to the DFA paper ALT test, as existing FDA-approved POC platforms for measurement of ALT in fingerstick blood (Roche Reflotron [28] and Alere

Cholestech LDX [29,30]) report no systematic bias or difference between capillary and venous blood. However, review of available information summarizing the performance of these two tests suggests that these tests were not actually validated with fingerstick samples from patients with elevated ALT values, but rather only with fingerstick samples from patients with normal to mildly elevated (e.g. maximum of 65 U/L) ALT values [28–30]. The systematic difference we observed between fingerstick blood and serum could easily have been missed if testing had only included patients with normal to mildly elevated ALT values. Given the absence of data comparing ALT levels in fingerstick vs serum samples in patients with elevated ALT, we had no apriori expectation of how well paper-based test results for these two sample types would agree. To our knowledge, this is the first study to evaluate the performance of a POC transaminase test on both fingerstick and serum samples in a patient population with a wide range of baseline ALT values. Unfortunately, our study was limited by the fact that the Alere Cholestech LDX was recalled by the FDA just prior to the start of this study, thus preventing the direct comparison between ALT results measured in fingerstick samples on the two platforms. Evaluation of the limited additional literature on these platforms is complicated by the use of different sample types, sample collection methods, and comparator methods. In an independent clinical trial report [31], Green et al compared serum ALT values measured by a Roche-Hitachi Modular Analyzer and paired whole blood ALT values measured by Cholestech LDX, and found that ALT values measured by Cholestech were systematically lower than those measured by Roche/Hitachi. However, another study by James et al [32] noted higher whole blood ALT values when measured on Reflotron compared to serum ALT values measured with a reference method. The authors observed excellent concordance of ALT results when whole blood and plasma were both analyzed using the Reflotron, and similarly when serum was analyzed by the Reflotron and reference method, supporting their conclusion that there was a disagreement between serum and whole blood values.

Further studies will be required to definitively conclude that there is an inherent difference in ALT concentration between venous and capillary blood. We note that the presence of a cover film on DFA paper devices used for testing whole blood was shown in prior experiments during device optimization to slow evaporation that otherwise reduced ALT values measured in whole blood compared to values measured in the same volume of serum with the same ALT concentration. Because use of the cover film for devices used with whole blood completely corrected the effect of evaporation on that sample type and equalized results obtained from whole blood and serum, devices used for fingerstick blood (but not serum) in this study similarly had a cover film. It is possible that the evaporation effect was not completely corrected for fingerstick blood, thus contributing to our findings of lower ALT values in fingerstick vs serum samples, but we find this unlikely. Regardless, the systematic difference we observed between DFA paper test results for fingerstick blood vs serum samples allowed us to calculate a correction factor that can now be applied to fingerstick results to allow them to match serum results obtained from the paper test (which in turn are in close agreement with automated platform results). Therefore, by using this correction factor to adjust the read guide, results of fingerstick testing with the DFA paper test will be calibrated to results of testing serum on the Abaxis Piccolo platform. Further clinical evaluation using the newly adjusted read guide will need to be performed in order to formally validate this correction factor.

This study has further demonstrated the feasibility of remote device reading, in which an image of the device and read guide captured by a simple cell phone camera can be texted to a central location and read there (on a computer screen) by a trained reader. Reads of both 2MP and 8MP camera images were in excellent agreement with the reads of the device in real time (Fig 4). This approach could allow the test to be performed without the need for a reader

familiar with device results interpretation to be present on site, and thus opens the door to home-based testing.

In conclusion, we have demonstrated excellent agreement between the DFA paper test and automated platforms for measurement of ALT, and shown that remote reading of the paper device is feasible. Our findings also shed important light on two key aspects of clinical ALT measurement: a) systematic differences in ALT levels by sample type (fingerstick vs serum), and b) variability in ALT measurements provided by different FDA-approved automated platforms. This POC device has significant global potential for monitoring for DILI in patients on potentially hepatotoxic medications, including those for TB and HIV.

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Author Contributions

Conceived and designed the experiments: SJ RR FN EC RS RB MC NA SK NRP. Performed the experiments: SJ RR EC RS SK NRP. Analyzed the data: SJ RR FN EC RS SK NRP. Contributed reagents/materials/analysis tools: SJ FN EC SK. Wrote the paper: SJ RR FN EC RS SK NRP. Assisted with patient recruitment: RB MC NA.

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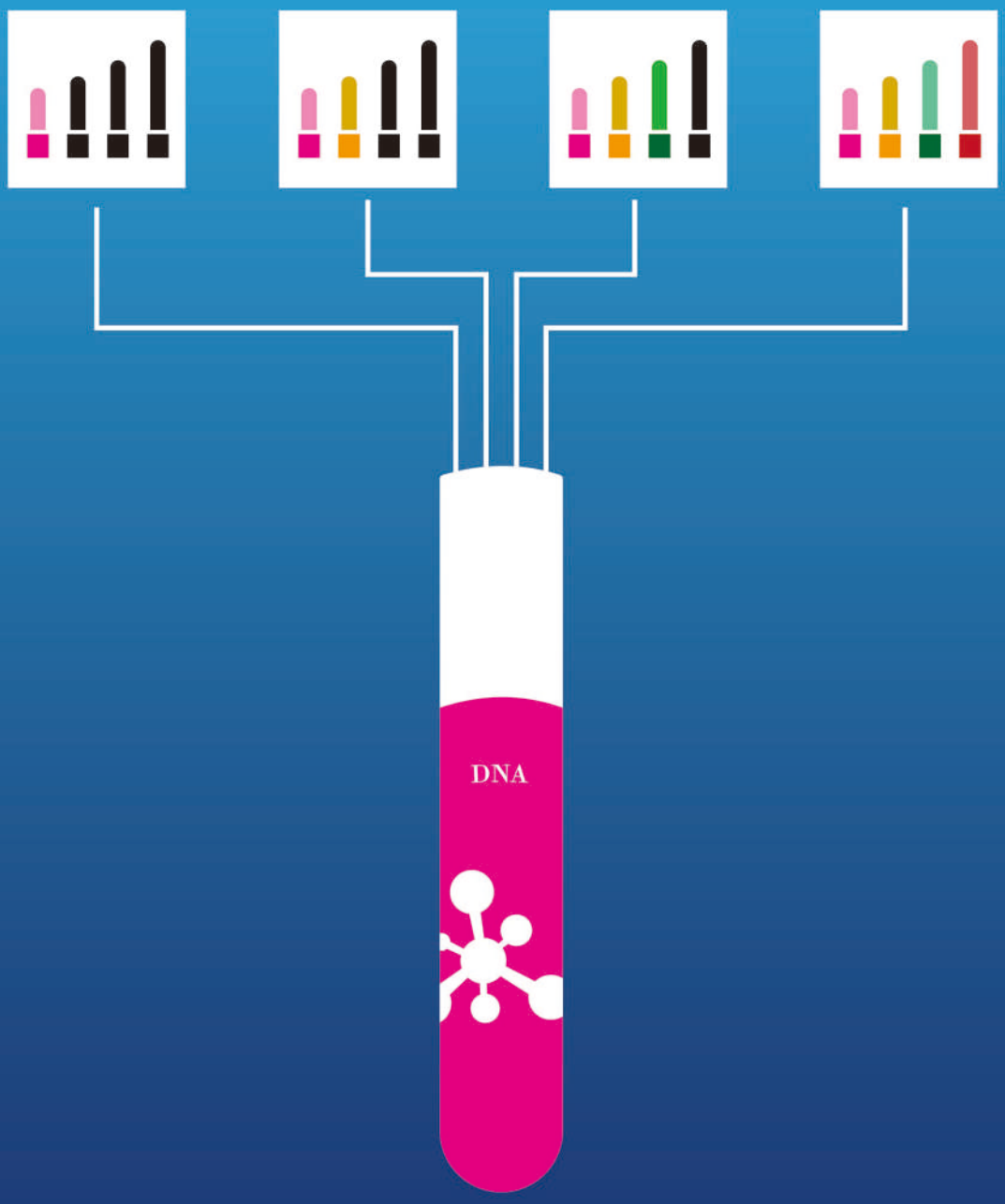

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