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Challenges of Development and Implementation of Point of Care Pharmacogenetic Testing

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Abstract

Introduction: Just as technology was the underlying driver of the sequencing of the human genome and subsequent generation of volumes of genome sequence data from healthy and affected individuals, animal, plant, and microbial species alike, so too will technology revolutionize diagnostic testing. One area of intense interest is the use of genetic data to inform decisions regarding drug selection and drug dosing, known as pharmacogenetic (PGx) testing, to improve likelihood of successful treatment outcomes with minimal risks.

Areas covered: This commentary will provide an overview of implementation research of PGx testing, the benefits of point-of-care (POC) testing and overview of POC testing platforms, available PGx tests, and barriers and facilitators to the development and integration of POC-PGx testing into clinical settings. Sources include the published literature, and databases from the Centers for Medicaid and Medicare Services, Food and Drug Administration.

Expert Commentary: The utilization of POC PGx testing may enable more routine test use, but the development and implementation of such tests will face some barriers before personalized medicine is available to every patient. In particular, provider training, availability of clinical decision supports, and connectivity will be key areas to facilitate routine use.

1. Introduction

Research into the genetic basis of adverse drug responses (ADRs) and drug response has resulted in the identification of many polymorphic variants resulting in altered protein function Testing for genetic variants associated with risk of ADRs or treatment failure, known as pharmacogenetic (PGx) testing, has become a pillar of the personalized (or precision) medicine movement. In many cases, PGx testing can take anywhere from 2 to 7 days or longer to complete (turnaround time), placing the provider in a difficult position to choose between delaying treatment, prescribing a minimal dose or alternative treatment not impacted by the gene, or monitoring patient response closely. With the increasing use of point-of-care (POC) tests for a wide range of clinical indications [1], the development of POC PGx tests could be used at the time treatment is required to alleviate treatment delays associated with PGx testing in centralized laboratories. Several nucleic acid-based POCs are

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under investigation for microbial and human genes using multiple platforms and thus, it is anticipated that POC PGx tests are on the horizon that will increase routine use of testing, reduce test turnaround time, and meet the goals of personalized drug treatment [2]. POC PGx tests can be used in a variety of settings where medications used from the emergency department to hospitals to mental health facilities to retail pharmacies. By having both the test result and knowledge of the patients medication needs, the result can be immediately interpreted and applied. In contrast, a hospital or reference laboratory providing PGx testing may not have access to the patient's clinical or medication record and thus, the test report will not likely include any specific recommendations.

However, the use of POC PGx tests will likely require some effort to implement due to both the novelty of the type of test as well as limited experience with POC tests in general in some clinical settings. In particular, provider preparation, lab certification, connectively, data management, and health information exchange are some of the issues to consider for the routine use of POC PGx tests. This review will consider the status of POC technology for PGx testing and the barriers to integration in provider and pharmacy settings.

2. Pharmacogenetic (PGx) Testing

Individual drug response can be difficult to predict as it is impacted by several factors including a patient's diet, co-morbidities, age, weight, drug-drug interactions, and genetics. Multiple genes are involved in key pathways in the absorption, distribution/transport, metabolism, and excretion of a drug [3]. In addition, with greater knowledge of the causes or mechanisms of physiologic pathways altered in disease states, increasingly more drugs are designed to target to a specific protein. Changes in the gene sequence encoding that protein can adversely impact drug binding and drug function and thus, testing of the target gene is required (also considered a PGx test but now referred to as a companion diagnostic test to be performed when a particular drug is indicated) [4]. More than 100 drugs include information about pharmacogenetics in the drug label [5] and 12 DNA-based PGx tests and 12 companion tests have been approved or cleared by the US Food and Drug Administration (FDA) (Table 1). However, many genetic tests, including PGx tests, are laboratory-developed tests (developed by individual laboratories), and do not require FDA approval or clearance (though draft guidance was issued in 2014 about oversight of laboratory-developed tests). The impact of potential new regulatory guidelines on laboratory-developed PGx testing is up for debate [6,7], but may unexpectedly stimulate development in the the POC PGx testing market.

Several hospitals have established PGx programs as a quality improvement or clinical research initiative. In general, two delivery models are emerging for PGx testing – testing may be ordered preemptively when a patient is healthy and results are stored in their medical records to be consulted at a later time when the patient requires treatment, or testing may be ordered when treatment is warranted. The University of Chicago's 1200 Patients Project offers enrolled outpatients taking one to six drugs a panel PGx test [8,9]. The PG4KDS research protocol at St. Jude Children's Research Hospital prospectively tests patients for a select group of PGx genes.[10] Vanderbilt University's PREDICT program also prospectively tests patients but through a quality improvement program for select PGX

genetic variants.[11] For treatment-indicated testing (ordered at the time treatment is needed), the pilot implementation project at the University of Florida added CYP2C19 to the test ordered pre-operatively for patients undergoing left heart catheterization [12]. While both delivery models are under investigation, the merits of each approach have been subject to some debate [13–15]. A major benefit of testing preemptively is to avoid any delay in treatment due to the time required to complete testing (test turnaround time). Although some laboratories can complete testing within 24 hours after receipt of the sample (therefore, likely minimum 48 hours), many PGx tests take between 3 to 7 days to complete, which may be an unacceptable length of time to delay treatment. However, the disadvantage to preemptive PGx testing is the need to store the test results in a way that can be checked each time a new medication is prescribed if an automatic alert system has not been developed to link medications, test results, and clinical recommendations.

3. Challenges of Point-of care (POC) test development

The major features of POC tests are their simplicity, stability of test reagents, demonstrated concordance with an established or accepted test, and overall device safety (equipment and reagents) [16]. There are several steps to consider in the POC testing process, from sample collection, to reagent stability, quantification or detection, results interpretation, and data reports [17]. Unlike for protein or metabolite-based POC tests, for nucleic acid-based POC tests, one of the major challenges is the need to consolidate three testing phases into a single device: 1) nucleic acid extraction; 2) amplification; and 3) detection [18]. The development of a nucleic-based testing device that is rigorous, portable, and relatively easy to operate has presented several challenges.

A POC test can address the problem of turnaround time when treatment is warranted in any type of health care setting. Delay of treatment may be only a few hours then, with a decision about drug selection or dosing made and electronically submitted to the pharmacy potentially before the patient arrives to pick up the medication. While several groups are working to develop rapid PGx tests to reduce turnaround time, these are still likely to be performed in a centralized laboratory and not approved for use as POC tests [19–25]. In the next section, some of the challenges of nucleic acid-based POC testing are described, followed by an overview of the current development of PGx tests, specifically rapid-based PGx tests, concluding with a discussion of some challenges to implementation of POC PGx tests in the clinic or other health care settings. As several reviews have been published about the different approaches under development for nucleic acid POC technologies [26–28], a cursory overview of each technology will be presented here with references for more detailed discussion of the subject matter.

3.1 Lab-on-a-chip (LOC)/Microfluidics

The popular lab-on-a-chip entails a multi-step system fitted to a single device that provides a rapid ‘sample to answer’ test. Microfluidics has been the predominant technology used for lab-on-a-chip devices, and has been successfully demonstrated for nucleic acid amplification testing for many conditions [29,30]. However, one major disadvantage is that they typically require additional equipment such as a pump or pressure to operate the device and thus, an

energy source [18]. However, new approaches are under development to minimize the equipment support needed to operate LOC tests. For example, Rodriguez et al. [31] recently reported on a paper-based (or paperfluidics) LOC device capable of nucleic acid extraction, amplification, and detection of human papillomavirus (HPV) 16 from cervical samples in less than an hour.

3.2 Paper-based Assays

A paper-based assay for nucleic acids offers a cheaper alternative to the more expensive chip-based platforms [18]. Several successful paper-based POC tests have been successfully developed for infectious diseases such as HIV, chlamydia, and influenza [32–34]. From specimen collection to detection, paper-based assays have been developed for each of phases of nucleic acid-based testing. For specimen collection, blood sample collection and storage has long used paper, illustrated with the successful use of newborn screening dried blood spots collected and stored on 903 filter paper over the past 50 years [35]. In addition, fast technology analysis (FTA) cards have been developed to lyse blood cells and denature damaging proteins, leaving the DNA bound to a fiber matrix, stable for long periods at room temperature. The cell lysing process takes about an hour and has been used for different extraction techniques [36]. These cards have been incorporated into nucleic-based POC assays for infectious disease [37].

For DNA amplification and detection, lateral flow assays (LFA) have been commonly employed, relying on capillary-driven movement of sample analyte and reagents on a dry porous material such as chromatography paper or nitrocellulose. Recently, Choi et al. [38] reported an LFA-based system for DNA amplification and detection that could be combined with a sample preparation system to create an integrated device.

3.3 Smart-phone-based POCs

Advances in the utility of smartphone devices as diagnostic tools have rapidly pushed this technology platform to the forefront of POC test development [39,40]. Particularly for home-based testing for disease surveillance and use in resource-limited areas, smartphone-based diagnostics presents a convenient and widely available tool to perform, store, and/or transmit test results. Several examples of protein-based testing using smartphone technology have been developed, but the challenges of nucleic-acid based testing have created a developmental lag. Use of smartphone fluorescence in a smartphone application was introduced in 2014 [41]. Recent examples include a single-step bioassays on serum or whole blood using smart phone, semi-conductor quantum dots to measure thrombin activity [42], a smartphone potentiometric biosensor to measure levels of salivary α-amylase, a biomarker of autonomic nervous system activity [43], and a hybrid microfluidic, smart-phone device for biomarkers associated with cardiovascular disease [44]. New smartphone-based molecular diagnostics are also beginning to emerge, such as the recent report on a device for diagnosis of the herpes simplex virus type-2 [45]. In 2012, Stedtfeld et al. [46] introduced the Gene-Z device that is operated with an iPod Touch and has since demonstrated the use of this device for parallel gene detections [47]. Combination with other techniques such as paper-based lateral flow assay with a smartphone-detection has been evaluated [48].

4. Challenges of Developing POC-PGx tests

PGx tests are anticipated to face similar challenges as other nucleic acid based-tests, with the major challenges being multiplex testing, reporting, and interpretation.

4.1 Nucleic Acid Extraction & Amplification

Genetic testing typically requires whole blood samples, but many devices have validated different specimens including saliva or buccal swabs. A new innovation with the FTA card is the development of FTA Elute cards that release DNA from the paper matrix with water, which have been shown to yield good quality DNA for analysis [49]. Following extraction, an amplification step is needed to generate a sufficient quantity of the specific gene region for detection. The most common technique for DNA amplification is polymerase chain reaction (PCR), but this requires a heat source and time, presenting substantial challenges to a POC (including the potential need for a power source). To reduce the time required of PCR, several isothermal approaches have been developed over the past several years for DNA amplification, including loop-mediated isothermal amplification (LAMP) and rolling circle amplification (RCA) [50,51]. Alternative approaches utilize microfluidics to facilitate a rapid-based (~6 minutes) PCR amplification protocol [52–55]. With paper-based systems, lyophilized reagents can be stably stored for long periods.

4.2 Multiplex testing

Unlike nucleic-based POC testing to detect the presence/absence of a virus/bacteria, multiplex testing is required to detect potentially two or more variants in a single human gene or virus (viral subtypes). Thus, for human genes with a single allelic variant, three types of test result are anticipated: homozygous for either the normal or variant allele or heterozygous. This complexity is further compounded by the presence of multiple alleles in the same gene or viral sample. Another issue for POC PGx testing is the potential need to assay multiple genes if relevant to decisions about treatment. At this time, many of the clinical PGx tests are single genes (e.g., CYP2C19 for treatment with clopidogrel), with one notable exception of the gene pair VKORC1/CYP2C9 for treatment with Coumadin. Thus, the challenge of multiplex analysis resides at the amplification stage, with the need to use multiple primer sets to amplify the targeted genes or gene regions of interest. Some lateral flow detection assays have successfully demonstrated detection of up to 13 human papillomavirus subtypes [56]. Litos et al. [57] reported a dipstick device to detect four alleles of the toll-like 4 receptor gene associated with the innate immune response system. The assay involved a single amplification step of the exon containing both SNPs and use of two allele-specific primers per SNP for a quadruple primer extension reaction in a 1.5 hour assay. The Gene-X device allows for parallel detection of multiple genes [46,47]. Use of shared or universal primer sets may reduce the complexity of multiplex assay [58]. Several multiplex biosensor technologies are being explored to improve test specificity and sensitivity for POC devices [59].

4.3 Nucleic Acid Detection

A number of detection techniques have evaluated to assess sensitivity and specificity including fluorescence-based, electrochemical, and nanoparticle-based assays [60,61].

Lateral flow assays (LFAs) have been a well-studied platform for nucleic acid detection, almost exclusively used for microbiology testing [62–64]. In addition, different types of conjugated probes have been evaluated for both proteins and DNA. For example, the presence of a target nucleic acid was easily visualized using a colorimetric change following hybridization with gold nanoparticles conjugated to an oligonucleotide probe [65]. For nucleic acids, a denaturation step is often required to enable binding with a conjugated, single-strand probe. Recently, Choi et al. [38] demonstrated a newly developed 1-hour POC test that combined DNA extraction and amplification with a LFA detection system using a layered-structure to separate the temperature and reagents required of each step. The newly integrated test was used to successfully detect *Streptococcus pneumonia* in blood and *E. coli* in a range of food samples with smartphone-assisted quantification of the colorimetric change.

4.4 Quality Control

As with any clinical laboratory test, quality control (QC) programs are an important part of POC tests to ensure accuracy and reliability. Internal QC and external quality assessment (EQA) are essential to assuring the accuracy of the test results. Professional guidelines require both an internal QC and EQA for POC tests [66,67]. The frequency of QC is often determined by several factors included the frequency of testing, test complexity, and the risk of an inaccurate result on patient outcomes [68]. Performance of a single test on a POC device will often utilize a disposable cartridge or set of reagents and thus, a quality control system cannot always identify performance issues that may arise per a test run. To address this issue, some POC devices are designed with QC systems such as calibration checks that are run as part of each test unit, assessing both the electronic or operating system as well as the reagents [69–71]. This also reduces the burden for clinical operators of the POC test that likely will have no training or background in laboratory medicine and thus, may not recognize the importance of performing of QC. Indeed, one of the issues identified by primary care practitioners regarding the use of a POC test was QC and training [72], EQA involves blinded testing of samples and comparison of the results from a laboratory's run to the known outcomes. Samples may be obtained from third parties (e.g., professional organizations) or split samples with a centralized laboratory [69].

Unlike a centralized pathology laboratory where most of the testing is performed on a single piece of equipment, several POC devices may be in use in a given clinical setting and thus, each will require QC and EQA. This creates a huge task for lab administrators to oversee that QC and EQA are regularly performed and documented for each device. Documentation of the performance of each POC device should include the dates of performance of QC, operator, results, and corrective actions (if warranted) [68,71] QC management software can greatly, particularly if multiple devices are used in one setting.

4.5 POC PGx Testing Under Development

Although rapid developments in POC technologies have occurred, many issues remain regarding the development accurate and easy-to-use devices and Implementation in a clinical setting. The anticipated merge between POC technology development and PGx knowledge presents an opportune time for POC PGx test development in the near future, both for

companion diagnostics and PGx tests [2,73]. For POC PGx tests, once the performance characteristics of the test have been established and shown to be comparably equivalent to standard testing protocols, assessing integration of the new device is a clinical setting to determine feasibility and utility would be the next step. However, evidence of clinical utility has lagged behind the discovery phase, resulting in a slower than anticipated uptake of PGx tests in clinical practice [74]. Therefore, the new device may be used in a clinical trial, enabling observations about the use of the test in a clinical setting as well as assessing the impact of the test result on health outcomes. For example, the use of a POC test developed by Spartan Biosciences known as the Spartan RX CYP2C19 was evaluated in a proof-of-concept study in a hospital-based setting [75]. Patients were randomized to receive the POC tests and if found to carry a CYP2C19 variant, would receive prasugrel or to the standard of care arm with no testing and treatment with clopidogrel. CYP2C19 carriers identified by the one-hour test were all confirmed by Sanger sequencing. A more recent trial with a similar study design of a testing arm/prasugrel and a standard of care arm/clopidogrel used an expanded POC test for 3 genotypes (CYP2C19*2, ABCB1 TT and CYP2C19*17) for patients undergoing PCI for ST-elevation myocardial infarction (STEMI) [76].

Dosing for one of the most commonly prescribed medication in the world, warfarin, is challenging due to myriad factors [77], often resulting in hospitalizations [78]. Some of the variation in drug response has been attributed to two genes, VKORC1 and CYP2C9, known to impact the efficacy (target) and metabolism of the drug, respectively [79,80]. Genetic testing for the two genes has been available for several years but test utilization has been low [81]. Knowledge of a patient's genotype is believed to be most beneficial for estimating starting dose [82], and therefore, a rapid genetic test is needed in order to consider a patient's genotype at the time of initial dosing decision. Recently, Zhuang et al. [83] developed an integrated, multi-SNP detection chip-based test that could be performed in about two hours on 0.5 microliters of blood. The integrated device consists of a paper-based nucleic acid extraction and PCR amplification chip combined with a reusable glass capillary electrophoresis chip. Detection is based on a colorimetric assay for two SNPs in the VKORC1 gene (−1639G>A and 1173C>T) and a single SNP in the CYP2C9 gene (the *3 allele- (1075A>C)).

However, differences in genetic variation by race or ethnicity have been documented in PGx research, indicating the need to develop POC tests that will include common variations from different populations in order for the test to be useful to all patients [84,85]. Disparities in the predictive value and clinical utility of PGx testing are illustrated by several studies (reviewed in [86], including a large randomized trial comparing the standard (clinical-based) for warfarin dosing to genotype-guided warfarin dosing [87]. This study reported that African Americans randomized to the genotype-guided arm did not fare as well compared to Caucasians participants; testing for the study was performed using one of two FDA-approved (non-POC) devices, the Gen-Mark Dx eSensor XT-8 or the AutoGenomics INFINITI Analyzer, both of which analyze CYP2C9*2 and *3 and VKORC1 3673 (−1639G>A) genotypes [87]. Another study reported that PGx-based dosing algorithms for warfarin may have reduced clinical validity for African-Americans due to the absence of genetic variants more commonly observed in this population.[88] Similarly, the prevalence of genetic variations associated with the CYP2D6 gene also ranges widely; the frequency of

variants associated with poor metabolism ranges from a high of 10 percent in patients of European ancestry to low of less than 2 percent in individuals of Asian ancestry.[89,90]

4.6 Regulatory Oversight

In the U.S., healthcare providers that wish to use POC tests in their office, laboratory, or other clinical setting must obtain certification from the state in compliance with the federal Clinical Laboratory Improvement Amendments (CLIA). Clinical tests are categorized into three major categories: high complexity, moderate complexity, and waived tests; POC tests are classified as waived tests. General categories of waived tests include general chemistry, general immunology, bacteriology, toxicology, and urinalysis. Although less than 1% of all laboratory tests are classified as waived, more than 254,000 health facilities are CLIA-certified to conducted waived tests, accounting for the majority of provider's offices that are CLIA-registered to conduct waived testing [91]. POC tests are considered medical devices and must be FDA-approved. In contrast, laboratory-based genetic testing is classified as high-complexity but are not currently required to undergo FDA review.

A handful of non-PGx POC nucleic acid-based tests have been approved by the FDA. California-based company Cepheid has several approved devices using its GeneExpert test platform. This rapid, multi-step test combines DNA extraction, amplification (PCR), and identification of target sequences into a single test cartridge and desktop device; testing can be completed in 1.5 to 2.0 hours for a range of tissue samples. However, no POC PGx tests have been approved or cleared by the FDA, but several nucleic acid-based PGx tests have. The FDA's *in vitro* diagnostics database for nucleic-based tests lists 12 PGx tests that have been cleared or approved by the FDA (Table 1; accessed May 3, 2016) and 12 companion diagnostic tests, or tests that must be performed to identify patients that meet the clinical indications for a specific drug. The same testing platform can be been used to market multiple PGx tests as has been done by Roche with their cobas® 4800 System for the detection of mutations in the KRAS, BRAF, and EGGR genes, each associated with response to cancer treatments [92].

Two U.S-based companies, Spartan Rx [93][93] and Verigene, have obtained FDA approval for the rapid analysis of genotypes for the CYP2C19 gene, best known for its association with the drug clopidogrel. Both tests are benchtop, closed-systems that combine the multi-step extraction, amplification, and identification with minimal operator involvement (sample collect, transfer to reaction tube, and initiate testing). The Spartan RX test can be performed in less than an hour and the Verigene test takes about 2.5 hours. However, the Spartan RX test currently only analyzes three SNPs in CYP2C19 (*2,*3, and *17), thus, limiting its utility to some populations. In July 2015, the Verigene device was recalled by FDA based on numerous reports of inaccurate test results. None of the PGx tests are categorized as 'waived' and not POC, therefore, their use is limited to CLIA-certified complex testing laboratories to be performed by trained laboratory providers. A handful of physician offices are certified to provide complex testing, but most of these types of test are performed in hospital or commercial-based centralized testing laboratories.

5. Challenges in Implementing POC PGx Testing

Regardless of the type of clinical setting, several barriers will need to be addressed to successfully perform and utilize results generated from POC PGx testing. A recent review of barriers to uptake of POC tests identified several barriers: 1) device performance and data management; 2) operational issues included staff training; 3) higher per POC test cost compared to lab-based per test cost; 4) regulatory issues and laboratory accreditation; and 5) overall economics of adoption [94]. Further discussion of the some of the issues that may pose challenges to adoption of POC PGx testing is provided in the next section.

5.1 Provider Training

Two key issues are relevant to the appropriate use and application of PGx POC tests—knowledge of PGx in general and knowledge and skill in the performance of POC tests. Several studies have reported providers' limited knowledge and/or experience with PGx testing [95–97]. A number of programs have been developed to address providers' knowledge gap, including experiential learning [98], clinical decision supports [99,100], continuing education [101], and curriculum development [102,103]. The greatest challenge with the use of POC PGx tests will likely be associated with test interpretation and understanding of how the results inform therapeutic decisions. Most likely, the results output will need to be linked with a clinical decision support program that can provide interpretation of the results for a given medication. Therefore, connectivity of the POC PGx device will be necessary until providers reach a level of confidence with genotype-guided treatment for the drugs they most commonly prescribe. Some current decision support programs include online calculators such as warfarindosing.org and https://www.pharmgkb.org/guideline/PA166104996 that consider patients' genotype (or potentially alert provider about availability of PGx testing for a newly prescribed medication) with other clinical biomarkers to estimate or predict risk for certain medications.

A survey of CLIA-waived settings reported that POC testing is primarily performed by nurses (46%) and medical assistants (25%), followed by physicians (9%) and staff with the equivalent of a high school degree (7%) [104]. Thus, a diverse group of health care workers are involved with POC testing with vastly different levels of training, skill and knowledge. With the exception of sample collection and initiation of the test, optimal POC tests are designed to require a minimal amount of effort and skill to operate, reducing error rates [71]. However, several studies have documented user errors with POC tests, mostly in the analytical phase, and these errors may potentially result in erroneous clinical care decisions (users [105–108]. As a result, more efforts have focused on providing support and training for POC users, particularly in resource-limited areas [71,107,109]. Although some providers receive general training in their curricula on POC testing as required by accreditation standards, as is the case for pharmacists [110], each test will have unique characteristics that warrant knowledge about the device before operating. Often, training to use specific POC devices is not time-consuming. For example, in a hospital-based study of POC testing for CYP2C19 to inform selection of anti-platelet medication, nurses completed a 30-minute training session to learn how to perform the test, which included sample collection (buccal swab), adding reagents to the device, and initiating testing (push-button) [75]. Another study

reported a half-day training of nurses to perform multiple POC tests related to care of HIV patients [111].

5.2 Integration of POC PGx Testing into Clinical Practice

In general, the slower than anticipated utilization of PGx testing may be due to several factors, including limited provider knowledge, lack of evidence of test utility, lack of clinical guidelines, and uneven coverage policies [74,112–115]. Similarly, POC tests in general have encountered some challenges to uptake, though for different reasons. While routine use of lab-based PGx testing appears to be stymied by the lack of evidence of clinical utility, routine implementation of POC tests is impeded by concerns with potential disruptions to workflow, training requirements, doubts of clinical value, cost, and evidence of equivalence to lab-based tests.

Optimally, POC PGx testing could be implemented in any type of clinical setting that provides prescription medications. For this discussion, we'll assume that POC PGx tests have been developed that meet the criteria for classification as a waived test by CLIA and FDA and therefore, do not require certification of compliance for high or moderate complexity testing and laboratory-trained personnel to perform the test.

Given the wide range of tests required for hospitalized patients, the hospital setting is experience with implementation of different POC tests have been gained the most. From routine monitoring of glucose levels [116], to emergency assessment of blood gases [117] or infectious disease [118], the wide range of devices used for different indications warrants establishment of POC manager or administrative team to ensure appropriate training and compliance [71]. In addition, the costs of use of multiple POCs must be considered to the hospital's own centralized laboratory services. However, in an office-based setting, space, staff training and practice acceptance are likely to present the key challenges. With a smaller setting and minimal resources, implementation of POC testing may meet greater resistance due to the need to obtain CLIA certification for waived testing, implementing and documentation of quality control and quality assurance tests, potential disruption to workflow, and increased cost per test as noted in a recent study of the barriers to uptake of a single POC test for C reactive protein by primary care practitioners [72]. However, experience with POC testing in rural or resource-limited care settings has been perceived as a valuable tool to improve the prompt diagnosis and treatment of patients and therefore, acceptable by providers in such settings [119]. Other evidence suggests that POC testing can be implemented with minimal disruption, reducing staff time related to follow-up with testing from a centralized laboratory and more importantly, demonstrated of improved health outcomes [120].

Based on CLIA statistics, pharmacies account for 4.2% of CLIA-waived laboratories (as of January 2016 update), although only 1% of pharmacists have reported conducting POC testing [104]. Although the reasons are unknown, a recent paper reported a precipitous decline, 15.9%, in the number of certified pharmacies between May 2015 and March 2016, down to a total of 9,110 laboratories, possibly due to new policies in Walgreens, one of the largest retail pharmacies [121]. In 2014, 14% of surveyed pharmacies reported conducting POC testing, highest among chain pharmacies [122]. POC testing for a range of infectious

diseases is available through community pharmacies [123]. The conceptual model for POC testing for infectious diseases in pharmacy settings proposed by Weber et al. [124] align with the delivery of PGx testing in a community pharmacy: for patients seeking treatment or with a prescription, rapid testing, and informed drug selection and dosing [124]. With the development of POC PGx tests, patient eligibility for testing could simply and quickly be determined by a prescription for a medication known to be impacted by a PGx variant. The test analyte, buccal swab, can be easily collected by a pharmacist or by the patient with pharmacist supervision, without requiring private rooms.

Pharmacists' experience with other POC tests may inform their use of POC PGx testing and anticipated problems. A recent review of pharmacists' use of POC testing for cholesterol found a positive impact on screening and identifying patients for referral for dyslipidemia [125]. In a health system, a pharmacist-operated anticoagulation clinic demonstrated reduced hospitalizations and increased time within the therapeutic range with a POC intervention [126]. For example, POC creatinine testing can be administered by the pharmacist to patients with unknown renal status impairment; consultation with the physician can lead to changes in medication dosing and improved outcomes [127]. Reimbursement for POC testing will likely remain a major issue for pharmacists, regardless of test type [125,128]. Since state pharmacy boards define the scope of practice for pharmacists, a published review of pharmacy regulations reported that fewer than 20 percent of states addressed POC [129]. In some cases, a collaborative drug therapy management (CDTM) agreement may specify pharmacists' scope of practice regarding testing, however, CDTM regulations in 12 states do not allow pharmacists to perform POC testing [129].

5.3 Connectivity/data management

With continuing implementation of electronic medical records, the need to communicate test results from POCT to a patient's record is critical [130]. The ability to store results in an efficient manner, identify trends, and integrate other data such clinical biomarkers and drug exposures, is essential for many POC devices, particularly for frequently performed tests. In hospital settings with potentially multiple POC tests and centralized laboratory tests performed for a given patient, the interface with the laboratory information systems are important to document and record test results in the patient's record. Studies have demonstrated the value of data exchange interfaces with medical records systems, reducing error and increasing efficiency [131–133]. The POC testing industry also recognized the need to incorporate connectivity capabilities in their devices and established the Connectivity Industry Consortium (CIC) in 1999 [134]. In addition to data exchange, data connectivity can provide clinical support, trouble-shooting, and completion of regular quality assurance and quality control measurements. The Clinical Laboratory Standards Institute issued its second update on POC connectivity in 2006 [135]. More recently, the U.S. Institute of Biomedical Imaging and Bioengineering (NIBIB) convened a workshop about systems engineering and POC testing, reiterating the continued importance of connectivity [136].

New molecular POC tests typically include data connectivity and other digital support [137]. Unlike POC tests for biomarkers that need to be continuously monitored (e.g., glucose) or

repeat testing (e.g., cholesterol) and infectious disease testing, POC PGx tests for DNA-based variants will be single use – the test result for a given gene will not change over the patient's lifetime. Therefore, the storage and portability of the result are critical to optimize consideration of the result each time a prescription is written and avoid duplicate testing. Data exchange between the clinical setting where the test is performed and the patient's pharmacy could improve the likelihood that the results are considered for every new prescription. Discussion of the PGx results with the patient, regardless if positive or negative, may also improve likelihood that information about testing will be shared with each treating provider.

6. Expert Commentary

As evidence of clinical utility for PGx testing continues to be generated through a variety of study designs (e.g., prospective, retrospective, cohort) in myriad clinical settings and countries, one of the potential factors that could impact outcomes is the timeliness of the information to be deemed clinical useful. Thus, reducing the test turnaround time to a few hours from several days is a key requirement to incorporating the information at the time treatment is warranted. Imagine the following scenario -- a patient visits their provider, a diagnosis is made, and treatment prescribed pending the outcomes of a PGx test that may impact dosing or drug selection. Or a provisional prescription is electronically submitted to the patient's pharmacy with instructions to not dispense until confirmed by the provider. A blood, buccal or saliva sample is obtained from the patient at the provider's office and testing initiated on a POC PGx device. By the time the patient arrives at the pharmacy, testing has been completed and the provider has reviewed the results and made any recommended changes to the prescription and communicated those changes (or confirmed that the original prescription is correct based on the patient's genotype) to the pharmacist. In addition, the PGx test results may be transmitted to the pharmacy to keep in the patient's record to confirm the safety and efficacy of future medications.

As discussed in the paper, the realization of this clinical scenario faces several challenges, both with the technical development of a POC PGx test and implementation of the test in various clinical settings. All of these issues can be addressed with careful development and preparation. The test's scope, number of variants, and gene complexity (i.e., pseudogenes) will all impact the selection of test platform, type/quantity of test reagents, QC, turnaround time, and cost. The scope of POC PGx tests may be a single gene with limited utilization (e.g., CYP2C19 in cardiology clinics) or multiple genes to accommodate a range of medications commonly prescribed (e.g., for patients in a general practice setting or urgent care setting). In addition, given the differing prevalence of some genetic variants between populations, it will be important to optimize the predictive value or benefit for all patients or otherwise note this important test limitation.

As POC PGx tests advance toward approval and introduction into clinical settings, collaboration between test developers and clinicians can help address some of the implementation challenges to facilitate adoption of these new tools in a clinical setting. As some devices may be implemented in different clinical settings, it is important for developers to gain a better of understanding of where the device potentially will be used and

by whom. Broad provider education will be essential, not just focused on physicians, but also nurses who may be more likely to actually be operating the POC PGx device. In addition, consideration of other groups involved in the use of POC PGx tests, namely patients and pharmacists, may also inform development of tools to promote patient understanding, acceptance, recall, and integration into pharmacists' review of potential risks associated with a new prescription (i.e., drug-gene interaction). Provider settings will need to obtain the appropriate CLIA certification to perform POC PGx tests. At this time, no waived POC PGx tests are FDA-approved, but technological advances will likely result in such tests in the next five years.

Furthermore, the implementation of POC PGx tests will likely differ between clinical settings, dependent on several factors including scale of testing, provider experience, training needs, acceptance and perceived value, and data connectivity needs. With the continuing growth in understanding of drug-gene interactions, it will be particularly important to develop or make accessible to a resource that can be easily updated for prescribers to use to interpret the test results and make the indicated medication adjustments. Development of and access to clinical decision support and patient resources will greatly facilitate the appropriate and informed use of POC PGx testing, respectively.

With the continued push for patient-centered care that is more personalized, decentralized, and accessible and the increasing number of POC tests, , a future with POC PGx testing is highly likely. Thus, there is an urgent need to begin to prepare health providers and health systems to integrate these new technologies into routine care.

7. Five year view

The field of pharmacogenetics has grown prodigiously in the past five years, and there is no indication of a drop-off in interest or funding of the genetic mechanisms of drug response or drug targets. Likewise, the continued technology development in microfluidics, nanotechnology, and smartphone-based POCs will lead to smaller, more rapid, and accurate devices. In addition, due to the mHealth movement and the use of various devices to track, store, and analyze personal data, patients will also play a greater role in collecting and providing data to inform health management and improve health outcomes once the issues of data sharing with providers and the electronic medical record can be resolved. In addition, more clinical offices may embrace the medical home model and include more POC tests to accommodate patient needs and improve patient satisfaction and convenience. In some cases, diagnosis may be made without a patient visit with the transmission of vital signs and other biomarkers with home-based testing. Thus, as with other industries, medicine will likely undergo a change in its practice model as more and more technologies are developed and adopted. POC PGx testing will become more routine in clinics, pharmacies, and potentially through home-based testing.

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Key issues

- Pharmacogenetic (PGx) testing entails the analysis of genetic variants associated with risk of adverse drug response, drug targets, and treatment failure.
- In many cases, PGx testing can take anywhere from 2 to 7 days or longer to complete (turnaround time), placing the provider in a difficult position to choose between delaying treatment, prescribing a minimal dose or alternative treatment not impacted by the gene, and/or monitoring patient response closely for adverse response.
- The desire for more rapid PGx tests to inform treatment decisions, development of nucleic acid-based point of care (POC) tests, early development of closed-system PGx tests, and the growing use of POC testing for a range of clinical settings suggest the imminent development of POC PGx tests.
- Technical challenges to the development of POC PGx tests include the test's scope (number of genes and gene variants) and gene complexity, which will inform selection of the appropriate testing platform.
- Implementation challenges in the adoption of POC PGx tests include provider experience/provider training, minimizing workflow disruptions, and connectivitySee comment in PubMed Commons below of the device to the health system/electronic records system.
- Clinical decision support and dosing algorithms that incorporate PGx test data will be essential tools to promote provider awareness and application of the tests results based on current evidence and clinical recommendations.

Table 1.

FDA Cleared or Approved PGx Tests (nucleic acid based-tests) (Excerpted from FDA Nucleic Acid based tests on 06/26/16 at (available at http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm) and List of Cleared or Approved Companion Diagnostic Devices (nucleic acid based-tests) (available at http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm).

PGx or Companion Diagnostic Test (Manufacturer)	Associated Medication	Genetic Variants Analyzed
Kit v3 (Luminex Molecular Diagnostics, Inc.)	Imatinib	CYP2D6 *1-*11, *15, *17, *29, *35, *41, and duplication genotypes
Spartan RX CYP2C19 Test System (Spartan Bioscience, Inc.)	Multiple	CYP2C19 *2, *3, *17
Verigene CYP2C19 Nucleic Acid Test (Nanosphere, Inc.)	Multiple	CYP2C19 *2, *3, *17
INFINITI CYP2C19 Assay (AutoGenomics, Inc)	Multiple	CYP2C19 *2, *3, *17
Invader UGT1A1 Molecular Assay (Third Wave Technologies Inc.)	Multiple	UGT1A1* 1 (TA6) and *28 (TA7) alleles
Roche AmpliChip CYP450 microarray (Roche Molecular Systems, Inc.)	Multiple	CYP2C19 *1, *2, *3 CYP2D6
eSensor Warfarin Sensitivity Saliva Test (GenMark Diagnostics)	Warfarin	CYP450 2C9 *2,*3 VKORC1 (−1639G>A)
eQ-PCR LC Warfarin Genotyping kit (TrimGen Corporation)	Warfarin	CYP2C9*2,*3 VKORC1 (−1639G>A)
eSensor Warfarin Sensitivity Test and XT-8 Instrument (Osmetech Molecular Diagnostics)	Warfarin	CYP450 2C9 *2,*3 VKORC1 (−1639G>A)
Gentris Rapid Genotyping Assay - CYP2C9 & VKORC1 (ParagonDx, LLC)	Warfarin	CYP2C9 *2 and *3 VKORC1 1173 C>T
INFINITI 2C9 & VKORC1 Multiplex Assay for Warfarin (AutoGenomics, Inc.)	Warfarin	CYP2C9*2,*3 VKORC1 (−1639G>A)
Verigene Warfarin Metabolism Nucleic Acid Test and Verigene System (Nanosphere)	Warfarin	CYP2C9 *2 and *3 VKORC1 1173 C>T
THxID™ BRAF Kit (bioMérieux Inc.0	Dabrafenib, Vemurafenib, Trametinib	BRAF V600E and V600K
cobas EGFR Mutation Test (Roche Molecular Systems, Inc.)	Erlotinib	exon 19 deletions and exon 21 (L858R) substitution mutations of the EGFR gene
VYSIS ALK Break Apart FISH Probe Kit (Abbott Molecular, Inc.)	Crizotinib, Ceritinib	LSI TP53 probe target (17p-)
COBAS 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc.)	Dabrafenib, Vemurafenib, Trametinib	BRAF V600E
VYSIS CLL FISH PROBE KIT (Abbott Molecular, Inc.)	Ibrutinib	17p-
KIT D816V Mutation Detection by PCR for Gleevec Eligibility in Aggressive Systemic Mastocytosis (ARUP Laboratories, Inc.)	Imatinib	*KIT* D816V mutation
PDGFRB FISH for Gleevec Eligibility in Myelodysplastic Syndrome/Myeloproliferative Disease (MDS/MPD) (ARUP Laboratories, Inc.)	Imatinib	5q31~33 gene rearrangement
cobas® EGFR Mutation Test v2 (Roche Molecular Systems, Inc.)	Erlotinib	Exon 19 deletions and L858R, G719X, exon 20 insertions, T790M, S768I and L861Q
therascreen® EGFR RGQ PCR Kit (Qiagen Manchester, Ltd.)	Erlotinib	exon 19 deletions and exon 21 (L858R)
cobas® KRAS Mutation Test (Roche Molecular Systems, Inc.)	Cetuximab, Panitumumab	seven somatic mutations in codons 12 and 13 of the KRAS gene

PGx or Companion Diagnostic Test (Manufacturer)	Associated Medication	Genetic Variants Analyzed
BRACAnalysis CDx™ (Myriad Genetic Laboratories, Inc.)	Olaparib	variants in the protein coding regions and intron/exon boundaries of the BRCA1 and BRCA2 genes
therascreen KRAS RGQ PCR Kit (Qiagen Manchester, Ltd.)	Cetuximab, Panitumumab	seven somatic mutations in the KRAS oncogene
therascreen EGFR RGQ PCR Kit (Qiagen Manchester, Ltd.)	Erlotinib Afatinib	exon 19 deletions and exon 21 (L858R) substitution mutations of the EGFR gene
INFORM HER-2/NEU (Ventana Medical Systems, Inc.)	Trastuzumab, Pertuzumab, Lapatinib, Everolimus	Her-2/Neu gene amplification
PATHVYSION HER-2 DNA Probe Kit (Abbott Molecular, Inc.)	Trastuzumab, Pertuzumab, Lapatinib, Everolimus	Her-2/Neu gene amplification
SPOT-LIGHT HER2 CISH Kit (Life Technologies, Inc.)	Trastuzumab, Pertuzumab, Lapatinib, Everolimus	Her-2/Neu gene amplification
HER2 CISH PharmDx Kit (Dako Denmark A/S)	Trastuzumab, Pertuzumab, Lapatinib, Everolimus	Her-2/Neu gene amplification
INFORM HER2 DUAL ISH DNA Probe Cocktail (Ventana Medical Systems, Inc.)	Trastuzumab, Pertuzumab, Lapatinib, Everolimus	Her-2/Neu gene amplification
HER2 FISH PharmDx Kit (Dako Denmark A/S)	Trastuzumab, Pertuzumab, Lapatinib, Everolimus	Her-2/Neu gene amplification