In this issue: POCT – making the point

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How could POCT be a useful tool for migrant and refugee health?

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ARTICLE

Point of care testing (POCT) represents an important step forward in the clinical management of patients. POC assays are easy to use and do not require skilled personnel; therefore they are particularly useful in low resource settings where diagnostics laboratories equipped with complex instruments that require well trained technicians are not available. Samples can be processed immediately overcoming the problems related to the stability of the sample, storage and shipping to a centralized laboratory hospital based.

Furthermore, results are delivered in real-time, usually less than 1 hr; thus, a clinical decision can be taken earlier. A prompt diagnosis is crucial in the case of contagious diseases allowing a rapid isolation of the infected patient and treatment; thus, reducing the risk of transmission of the pathogen.

In this report, we address the use of POC assays in the diagnosis of infectious pathogens including hepatitis B and C viruses, human immunodeficiency virus-type 1, human papillomavirus, chlamydia trachomatis, neisseria gonorrhea, trichomonas vaginalis, mycobacterium tuberculosis and the parasite plasmodium. These pathogens are commonly detected among vulnerable people such as refugees and migrants. The described POC assays are based on nuclei acid amplification technology (NAAT) that is generally characterized by a high sensitivity and specificity.

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INTRODUCTION

In recent years, conflicts in Syria, Afghanistan, Iraq, and persecutions in South-East Asia (i.e, Rohingya in Myanmar) or sub-Saharan countries forced millions of people to leave their countries because of the economic crisis (economic migrants) or the fear of consequences if they remained in their own country (refugees). The destination is usually in neighboring countries that host the majority of refugees, but also high-income countries such as Germany, Italy, France [1]. In the last seven years, Turkey hosted the largest refugee's population with almost 4 million refugees and asylum seekers including 3.6 million Syrians and almost 330,000 people from other nations [2]. Another 1.4 million Syrians hosted in refugee camps in Jordan [3].

In Italy, during 2020, we assisted at a continuous increase in the arrivals due to the conflict in Libya [4]. International agencies, non-government organizations and governments support this vulnerable population through programs for education, healthcare, psychosocial support and other needs. The COVID-19 pandemic with its dramatic socioeconomic consequences and the impact on the health system of the affected nations, made even more critical the conditions for refugees and migrants, which already have a limited access to healthcare. Living in camps facilitates the spread of transmissible agents such as mycobacterium tuberculosis or SARS-CoV-2 with an increase in morbidity and mortality. Regarding SARS-COV-2, it has been reported that the transmission rate is high in the shelters [5], and this in turn exposes the local community to a higher risk of COVID-19. Monitoring of the health conditions of refugees and migrants within the camps is not an easy task because of the lack of clinics and hospitals. Point-of-care testing (POCT) could be a valid alternative to laboratory hospital-based testing. Furthermore, POCT does not require highly skilled and trained personnel, and overcomes the problems related to specimen stability and quality of the analytical results. Samples can be processed immediately and results delivered in real-time providing a reliable solution to the errors that may occur in the pre-analytical phase.

Here, we describe the most updated point-of-care (POC) assays using the nucleic acid amplification technology (NAAT) for the diagnosis of some common infectious diseases including viral hepatitis B and C, human immunodeficiency virus type 1 (HIV-1), sexually transmitted diseases, tuberculosis, COVID-19, and malaria.

POC ASSAYS FOR THE DIAGNOSIS OF VIRAL HEPATITIS B AND C AND HIV-1

Infection by hepatitis viruses B (HBV) and C (HCV) can be detected in serum or plasma samples by quantitative real-time PCR. The majority of the available assays are designed for batch testing of multiple specimens within a run (6-9). The Xpert® HBV Viral Load test and the The Xpert® HCV Viral Load test (Cepheid, Sunnyvale, CA, USA), on the contrary, may be run on-demand and deliver results in 90 and 105 min, respectively. The systems require a single use disposable GeneXpert cartridge, which contains

all reagents needed for sample preparation, nucleic acid extraction and quantification of PCR product. The Xpert® HBV Viral Load test has a dynamic range of quantification of 10^1 to 1×10^9 IU/mL (1.0 to 9.0 Log IU/mL), with a limit of detection (LOD) of 3.20 IU/mL for plasma and 5.99 IU/mL for serum according to the manufacturer's product package. The viral genome region targeted by the primers and probe set is the preC-C (Pre-Core-Core) gene. The assay detects the HBV genotypes A to H. Comparative studies showed the good performance of the Xpert® HBV Viral Load test when compared to other validated assays used for monitoring chronic HBV patients [10-14].

The Xpert® HCV Viral Load test quantifies HCV RNA within a range of 10 to 100,000,000 IU/mL and is validated to detect HCV genotypes 1 to 6. LOD is 4.0 IU/ml for EDTA plasma and 6.1 IU/ml for serum. It performed equally well when compared to the Abbott RealTime HCV viral load similarly to the Xpert HCV Viral Load Finger-Stick POCassay [15].

The Xpert® HIV-1 viral load assay allows the measurement of HIV-1 RNA in plasma in 91 min using a single cartridge for RNA extraction, purification, reverse transcription and cDNA real time quantitation. Test can be run on demand. Considering the very high number of infected individuals in the sub-Sahara African region, this molecular tool may be useful for monitoring infected individuals on therapy and whenever is required an urgent testing [16, 17]. The linear range of quantification of the assay is comprised between 40 to 10, 000, 000 copies/ml, and detects HIV-1 groups M (subtypes A, B, C, D, AE, F, G, H, AB, AG, J, K), N and O.

Instead, Xpert® HIV-1 Qual is designed to detect acute infection in high-risk population or vulnerable subjects. The test can be run on dry blood spot (18) or whole blood and delivers result in 93 min. There is a high correlation with widely used validated molecular assays [16-18] (Table 1).

POC ASSAYS FOR THE DIAGNOSIS OF SEXUALLY TRANSMITTED INFECTIONS

Sexually transmitted infections are still a significant global health problem, especially in developing countries where infections by human papillomavirus (HPV), chlamydia, gonorrhea, syphilis and trichomonas vaginalis are widespread [19]. POC testing can be a useful approach to identify quickly infected people. There are several HPV point-of-care testing platforms in the market such as the *care*HPV test (Qiagen, Hilden, Germany), but only the Xpert® HPV has been validated for the rapid detection of 14 high-risk HPV types [20] reported as HPV16, HPV18/45 or other high-risk HPVs (31, 33, 35, 52, 58; 51, 59; 39, 56, 66, 68). Time to result is 60 min. On the same platform can be run rapid tests for chlamydia trachomatis (CT), neisseria gonorrhoeae (NG) and *trichomonas vaginalis* (TV), which provide result in 90 and 40 min, respectively.

Other POC assays for CT/NG include the io CT/NG Assay (binx health, Inc) that uses a specific single use cartridge where the sample is directly loaded. Time to result is 30 min and the amplified product is detected by hybridization of the labeled probe and cleavage of the label [21]. Trichomonas vaginalis can be detected also by AmpliVue assay, Aptima, and Solana POC assays [22]. AmpliVue assay uses isothermal helicase-dependent amplification (HAD) and targets a conserved repeat DNA sequence of TV. The amplified product is visualized by lateral-flow strip-based colorimetric detection in a disposable device. The turnaround time is about 45 min. The assay showed a sensitivity of 100% and a specificity of 97.9%–98.3% when compared to microscopy or culture methods [22]. Compared to reference standard Aptima TV NAAT assay,

Table 1 Validated POC assays for the detection of HBV, HCV, HIV-1

Molecular assay	Specimen type	Target gene	Limit of detection (LOD)	Linear range	Time to result	Company
Xpert® HBV Viral Load test	Plasma EDTA or serum	Pre-Core-Core	3.20 IU/mL in plasma and 5.99 IU/mL for serum	10 to 1 × 10^9 IU/mL	< 60 min	Cepheid, Sunnyvale, CA, USA
Xpert® HCV Viral Load test	Plasma EDTA or serum	5'Untranslated region (UTR)	4.0 IU/mL in plasma and 6.1 IU/mL in serum	10 to 1 X 10^8 IU/mL	105	Cepheid, Sunnyvale, CA, USA
Xpert® HCV Viral Load Finger-Stick test	Whole blood	5'Untranslated region (UTR)	22 IU/mL genotype 1a, 35 IU/mL genotype 6e	100 to 100^8 IU/mL	< 60 min	Cepheid, Sunnyvale, CA, USA
Genedrive® HCV ID Kit	Plasma EDTA	5'Untranslated region (UTR)	Sensitivity at the detection threshold of 12-16 IU/ml	NA*	90 min	Genedrive Diagnostics Ltd, Manchester, UK
Xpert® HIV-1 viral load test	Plasma EDTA	ND*	18.3 cp/ml (WHO reference material); 15.3 cp/ml (VQA reference material)	40 to 10 6 copies/ml	91 min	Cepheid, Sunnyvale, CA, USA

**NA: Not applicable; ND: Not declared.*

the sensitivity of the AmpliVue was 90.7%, and the percent of agreement was 97.8% (Cohen's kappa=90.7) [23]. Solana TV assay is a qualitative PCR, which targets a conserved repeat DNA sequence of the microorganism using the HAD technology. The assay showed a high sensitivity and specificity in detecting TV in swabs and urine of both symptomatic and asymptomatic women when compared to FDA reference standards. Compared to the Aptima TV assay the sensitivity/specificity performance was 89.7%/99.0% for swabs and 100%/98.9% for urines [22]. Finally, Xpert TV showed a high sensitivity and specificity when testing vaginal swabs (96.4%/99.6%), endocervical swabs (98.9%/98.9%) and urine (98.4%/99.7%) [22] (Table 2).

Table 2 POC assays for sexually transmitted diseases

Molecular assay	Specimen type	Target gene	Time to result	Company
Xpert® HPV	Cervical cells	E6/E7	60 min	Cepheid, Sunnyvale, CA, USA
Xpert® CT/NG	Cervical, vaginal, rectal, and pharyngeal swabs; urine	gDNA (one)/ gDNA (two independent)	90 min	Cepheid, Sunnyvale, CA, USA
Xpert® TV	Vaginal and endocervical swabs; urine	*gDNA*	40 min	Cepheid, Sunnyvale, CA, USA
io CT/NG Assay	Vaginal swab; urine	gDNA (one)/ gDNA (two independent)	30 min	binx health, Inc, MA, USA
AmpliVue Trichomonas assay	Vaginal swab	Conserved repeat DNA sequence	45 min	Quidel, CA, USA
Solana TV assay	Vaginal swab; urine	conserved repeat DNA sequence	< 40 min	Quidel, CA, USA

POC ASSAY FOR THE DIAGNOSIS OF TUBERCULOSIS

Early detection of mycobacterium tuberculosis (MTB) is of paramount importance for improving patients' management and to reduce the risk of infection transmission. A POC assay endorsed by the WHO (http://www.euro.who.int/__data/assets/pdf_file/0006/333960/ELI-Algorithm.pdf) that responds to this need is the Xpert® MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA). The assay detects MTB and rifampicin resistance simultaneously in less than 80 min, and can resolve quickly those cases with a negative smear at microscopy [24]. The system is easy to use and is highly sensitive (11.8 cfu/ml) and the probes target the *rpoB* gene (Cepheid | MTB/RIF Molecular Test - Xpert MTB/RIF Ultra).

POC ASSAYS FOR THE DIAGNOSIS OF SARS-COV-2

Nowadays, emergency department are overcrowded by patients who present at admission with respiratory symptoms suggestive of coronavirus disease 2019 (COVID-19) caused by the recently uncovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These patients are confined into dedicated areas in order to limit the risk of viral transmission to negative patients until the result of the PCR becomes available. With the currently available platforms, results are not available before 4-5 hr from the arrival of the sample in the laboratory. In order to speed up the diagnostic process, to reduce the risk of transmission within the hospital, and to start treatment earlier, POC assays may represent a valid diagnostic alternative. Actually, POC assay on average deliver results in less than 1 hr [25], and have a high sensitivity and specificity [26]. We have recently demonstrated an excellent level of agreement between the Allplex™ SARS-CoV-2 assay and the POC VitaPCR™ SARS-CoV-2 assay [26], confirming the utility of POCT testing in the screening of suspected COVID-19 patients [27]. A list of some POC assays currently available on the market is reported in Table 3.

POC ASSAYS FOR THE DIAGNOSIS OF MALARIA

Malaria is a global health problem and its eradication is one of the major goals of the WHO. Five plasmodium species are known to infect humans: P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi [28]. Severity of the disease may vary in relation to the plasmodium species; therefore, a correct identification is important for the best therapeutic approach as well as an high sensitivity. A sensitive assay is important for the identification of asymptomatic carriers that are potential infectious reservoir for the plasmodium spread [29]. Although validated assays are not available yet, efforts have been made to construct portable PCR machines that could be used near the patient [30-33]. A promising platform consists of a disposable plastic chip and a portable real-time PCR machine. The chip contains a desiccated hydrogel with the reagents needed for the identification of plasmodium by PCR. The chip can be stored at room temperature and rehydrated on demand with unprocessed blood [33].

CONCLUSIONS

POC diagnostics is becoming increasingly important within health service systems either in developing and advanced countries. The short turnaround time, the high specificity and sensitivity, the possibility to use unprocessed samples and the easy-to-use improved patients' management reducing the time of diagnosis and accelerating treatment. In the hospital settings, this translates in a more rapid transfer of the patient from the emergency department to the most appropriate clinical area. In the case of shelters where are hosted refugees or immigrants, POCT allows screening of numerous people in real-time without the need of shipping the samples to centralized hospital-based laboratories. The availability of test results in real-time allows to take immediate clinical decisions without delays with positive effects on the individual health. Vulnerable people that live in remote areas or hosted in camps or shelters will be those who benefit most from this new technology. The assays described allow for testing one or multiple infectious agents. Decision to test for one or multiple infectious agents is made based on the triage questionnaire administered to the patient.

A limitation of this new technology is represented by the cost of the assay, which is usually higher of a standard laboratory assay. A politics aiming at lowering the price of such reagents would facilitate their use in low resource settings with benefits for the local population.

Table 3 A short list of POC assays validated for the detection of SARS-CoV-2 RNA in respiratory samples

Molecular assay	Specimen type	Gene target	Limit of detection (LOD)	Time to results	Company
Xpert Xpress SARS-CoV-2	nasopharyngeal, nasal, mid-turbinate swab	E, N2	250 copies/ml	45 min	Cepheid, Sunnyvale, CA, USA
QIAstat-Dx Respiratory SARS-CoV-2 Panel	Nasopharyngeal swab	E, ORF1b, RdRp	500 copies/ml	60 min	Qiagen, Hilden, Germany
BIOFIRE® Respiratory panel 2.1	Nasopharyngeal swab	S, M	160 copies/ml	45 min	bioMérieux, Marcy l'Etoile, France
Simplexa™ COVID-19 Direct kit	nasal swab, nasopharyngeal swab, nasal wash/aspirate, and bronchoalveolar lavage	ORF1ab, S	500 copies/ml(NPS, NW/A) 242 copies/ml(NS): 1208 copies/ml (BAL)	> 60 min	DiaSorin Molecular LLC, Cypress, CA, USA
VitaPCR™ SARS-CoV-2 Assay	Nasopharyngeal swab, oropharyngeal swab	N	2.73 copies/µl	20 min	Menarini Diagnostics, Florence, Italy
ID NOW COVID-19 assay	Nasopharyngeal swab, throat swab	RdRp	125 genome equivalents/ml	13 min	Abbott Diagnostics, IL, USA

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