3차원 종이칩만으로 현장에서 감염병을 찾아낸다

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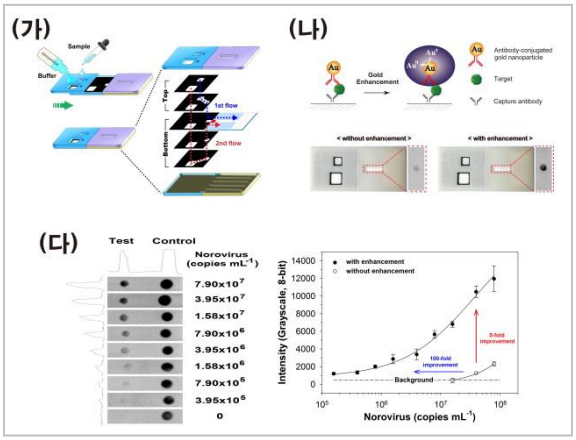
연구내용

선진국형 식중독으로 알려진 노로바이러스 감염 여부를 현장에서 바로 확인할 수 있는 종이형태의 검출/진단 키트가 개발됨에 따라 현장진단 분석 플랫폼 제작의 원천기술이 확 보되어 향후 다른 전염병을 현장에서 확인하 는 새로운 검출/진단키트 개발이 가능해짐. 연구팀은 자체 단독 연구를 통해 종이칩 (paper chip)을 이용한 고감도 저비용 현장진 단(POC) 분석 플랫폼*의 원천기술을 확보하 고, 인체감염형 노로바이러스에 적용하여, 30 분이내에 오염 여부를 확인하는 기술 개발에 성공함. 친수성 · 소수성 왁스 패터닝 및 수직 적층 배열 · 구성을 통해 저비용 소재인 종이 표면에 3차원 유체유로를 형성하고, 간단한 슬라이딩 조작을 통해 비전문가도 손쉽게 고 감도 데이터를 얻을 수 있는 새로운 원스텝 현장 진단키트를 개발함.
이번에 개발된 진단키트는 금나노입자의 촉 매특성을 활용한 금속 침전법을 이용함으로써, 단순한 조작 하나만으로도 검사의 측정감도를 획기적으로 증폭시켰으며, 기존 상용 노로바이러스 신속진단키트의 단점으로 지적되던 검사 민감도를 최대 100배 향상시키는데 성공함.



[그림1] (가)신속현장진단키트의 구성 및 작동 원리 모식도 (나) 금나노입자 촉매특성을 이용한 측정감도 증폭 원리 및 증폭 결과 이미지 (다) 농도별 노로바이러스 검출 이미지 및 기존 방법과의 검출결과 비교 그래프

기대효과

POC 검사/분석 분야는 대형의료기기 시장과는 달 리 아직까지 세계적인 절대 강자가 없는 분야이므로, 이번 연구를 통한 플랫폼에 대한 원천기술 확보를 계기로 향후 타 출연(연)과의 융합연구를 통하여, 지카, 메르스, 뎅기열바이러스에 대한 고감도 검출 장치개발에 나설 계획이며, 전염병과 같은 생물재난에 대한 국가사회 문제해결을 위한 연구를 통하여, 거대 다국적 기업들과 맞설 수 있는 기술 경쟁력을 선점하는 기회를 제공할 것으로 기대됨.

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Three-dimensional paper-based slip device for one-step point-of-care testing

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In this study, we developed a new type of paper-based analytical device (PAD), the three-dimensional (3D) slip-PAD, to detect infectious human norovirus for global healthcare. The 3D configuration of the papers combined with a slip design provides unique features and versatility that overcome the limitations of fluidic manipulation and sensitivity in point-of-care (POC) tests. The assay can be carried out in a single step based on a moveable slip design, making it suitable for unskilled users. The 3D fluidic network developed by layered construction of wax-patterned papers provides different fluidic paths for the sequential delivery of multiple fluids without the need for peripheral equipment. The release and mixing of enhancement reagents on the device improved the sensitivity and detection limit. The assay results could be visualized by naked eye within 10 min, with subsequent amplification of the signal over time (<60 min). The device showed a broad dynamic range of detection and high sensitivity, with a detection limit of 9.5×10^4 copies ml^{-1} for human norovirus. These results demonstrate that the 3D slip-PAD is a sensitive diagnostic assay for detecting human norovirus infection that is particularly suitable for POC testing in regions where resources are scarce.

There is an increasing demand for point-of-care (POC) tests in many fields, including clinical analysis, food safety, and environmental assessment, and many efforts have been made to develop biosensors that are affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users (ASSURED) that meet World Health Organization criteria[1–5]. Paper-based analytical devices (PADs) have received considerable attention for POC applications owing to the advantages of low cost, light weight, ease of handling (e.g., folding, cutting, patterning, etc.), and fluid transport by capillary wicking[6–8]. In particular, a lateral flow assay (LFA) based on PADs is commercially available for a variety of applications that is low cost, rapid, simple to use, equipment-free and easily mass produced, thus conforming to ASSURED criteria[8]. However, it also has limitations in terms of sensitivity and manipulation of fluid flow.

To overcome these drawbacks, recent research on PADs has focused on improving detection signal and modifying paper geometry. For example, various detection labels [nanoparticles (NPs), carbon nanotube, quantum dots, fluorescent dyes, etc.] and amplification methods (enzymatic reaction and metallic precipitation) have been used to improve detection signal and sensitivity[9–13]. However, these do not meet the growing demand in sensitivity, and require multiple amplification steps (successive addition of reagents and washing) for optical or electrochemical detection. On the other hand, PADs with different types of geometry [three-dimensional (3D), folding, and slip, etc.] have been developed to manipulate fluidic flow and to retain operational simplicity[14–20]. A 1D lateral flow can be extended to 3D flow by assembling papers with hydrophilic/hydrophobic channels, which enables the control of liquid flow in a 3D fluidic network. Nonetheless, developing a one-step device that affords sequential delivery of multiple fluids for detection in a single step remains a challenge.

Norovirus is a highly infectious pathogen that is the major cause of nonbacterial epidemic outbreaks of gastroenteritis around the world[21]. According to the Centers for Disease Control and Prevention, norovirus causes 19–21 million infections, resulting in 56,000–71,000 hospitalizations and 570–800 deaths each year in the United States. The virus can be transmitted by ingestion of contaminated food or water, person-to-person contact, and contact with contaminated surfaces or objects. There are currently no vaccines or antiviral drugs for the treatment of norovirus infection; therefore, a rapid and sensitive detection system is essential for preventing and reducing norovirus outbreaks in both developed and developing countries. There are several methods for detecting norovirus in humans, including electron microscopy, enzyme-linked immunosorbent assay (ELISA), real-time reverse

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