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Review

Culture-independent diagnostic testing: have we opened Pandora's box for good?

J. Michael Janda [a,*], Sharon A. Abbott [b,1]

[a] Public Health Laboratory, Division of Communicable Disease Control and Prevention, 1000 Broadway, Oakland, CA 94607, USA
[b] Microbial Diseases Laboratory, California Dept. of Public Health, 850 Marina Bay Parkway, Richmond CA 94804, USA

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ABSTRACT

The ability to accurately and quickly identify microbial agents associated with infectious diseases has been a longstanding and continuous goal of diagnostic microbiology laboratories. Over the course of several decades, technology and testing methodologies in this field have gradually evolved from traditional- or classic-based culture and identification approaches to antigen capture systems and more molecular-oriented applications. Recently, these molecular-based applications have signaled a new era in clinical diagnostic microbiology with the commercial introduction of culture-independent diagnostic testing (CIDT) systems. The first major commercial venture into the CIDT arena involves the detection of acute bacterial gastroenteritis. Several commercial products are now on the market globally with at least 4 Food and Drug Administration approved since January of 2013. These new systems offer the direct detection of a variety of enteropathogens quickly without the need for traditional culture. In Greek mythology, Pandora opened a "jar" or "box" out of curiosity thereby releasing all of humanity's evils most notably diseases and plagues according to Hesiod's *Theogony*. While not ill-intentioned the only thing left in the box was Hope.

1. Introduction

The ability to provide rapid, highly accurate microbial identification, thereby potentially impacting clinical diagnosis, treatment, and medical prognosis, is one of the major challenges continually facing clinical microbiology laboratories. In the 1960's and early 1970's, this goal was fraught with numerous technical difficulties and challenges as conventional biochemical testing and culture, even for rapid growers, was a time consuming and laborious procedure and test results for many common pathogens required 48–72 h incubation before final reports could be issued. The "gold standard" for test results during this era was the successful culturing and identification of recognized pathogens from contaminated or sterile body sites (Kronvall and Larsson, 2007). A perfect example of such an approach involved the detection of syndromic diseases including infectious diarrhea. Laboratory practice at that time necessitated culture (bacteria), stains (parasites), and electron microscopy (viruses) to detect the most common agents associated with gastroenteritis based upon symptomatology (Turgeon and Fritsche, 2001). Other comparable scenarios included laboratory workups for bloodstream infections, respiratory tract, and sexually transmitted diseases (STDs).

2. Historical

Approaches to the diagnosis of various infectious diseases in the microbiology laboratory began to change in the late 1960's and early 1970's with the introduction of a number of technological developments including development of the microtest format, miniaturization of multiple test systems, reduction in biochemical inoculations and test reagents, resulting in a quicker turnaround time of 18–24 h (Janda and Abbott, 2002). Two of the most notable of these microidentification systems originating in this era were the Enterotube II (Becton-Dickinson, Sparks, MD, USA) and the API® 20E (bioMérieux Inc., Durham, NC, USA) test strips. During the mid-1970's, these products and others revolutionized the identification of facultatively anaerobic gram-negative bacteria as well as other similar groups (Butler et al., 1975; Grunberg et al., 1969). The changes pioneered by these systems continued to evolve as technology made great strides in the detection of infectious agents. Fig. 1 depicts some of these noteworthy changes from 1960 to 2010. Subsequently, the first part of the 1980's witnessed numerous changes that revolved around automated identification/susceptibility systems like MicroScan® (Siemens Medical Solutions, Malvern, PA, USA), Vitek® (bioMérieux), and standalone enzyme-linked immunosorbent assay-based methodologies such as Gonozyme® and Rotazyme® by Abbott Laboratories (North Chicago, IL) for the detection of STDs or viruses. By the 1990's, platforms began to change from phenotype expression (API®-20E, MicroScan Vitek®-2; BiOLOG panels, Hayward, CA, USA) to molecular approaches with the development of PCR-based

* Corresponding author. Tel.: +1-510-268-2705.
E-mail address: Michael.Janda@acgov.org (J.M. Janda).
[1] Retired.

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reactions and first-generation sequencers using common housekeeping genes such as 16S rRNA to achieve microbial identifications. Today, the molecular field has exploded to include multiple platforms including second-generation (pyrosequencing) and third-generation (Ion Torrent) sequencing, mass spectroscopy–based applications (matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) and electrospray ionization (ESI-MS)), and microarray analysis (Glenn, 2011). Collectively, these technologies not only provide quicker and more accurate test results but can also provide microbial identifications of non-viable infectious agents from residual nucleic acid present in various human fluids or tissues such as blood and serum.

3. The concept of culture-independent diagnostic testing (CIDT)

While the terms "culture-independent diagnostics" or "nonculture diagnostic tests" may be relatively unfamiliar to many microbiologists, the practice itself of CIDT has been around for decades (Atkinson et al., 2013; Cronquist et al., 2012; Jones and Gerner-Smidt, 2012). From the infancy of diagnostic microbiology to its present-day format, a variety of techniques have been utilized to detect various infective agents without the use of standard culture. One of the chief reasons for the multitude of approaches is the inability to routinely culture many microbes. Examples include those associated with STDs (*Treponema pallidum*, chlamydia, and HIV), unusual bacterial or parasitic diseases such as leptospirosis and toxoplasmosis, rickettsia, zoonotic illnesses including Lyme disease and rabies, and many other viral-associated syndromes. Such infections were commonly diagnosed by age-old techniques including immunoserology and light and electron microscopy of stained tissues and fluids (Curry, 2000). Second, CIDT has been a mainstay for years in the form of serologic testing where exposure, subclinical infections, and convalescence or immune status can often be detected easier from serum than by routine culture. This includes such things as brucellosis or exposure to tuberculosis through QuantiFERON® testing. CIDT also made major strides in direct detection without culture for detecting important public health pathogens in developing nations or rural areas of the globe where laboratory support is non-existent. Examples here include rapid identification of cholera without culture by dipstick methods such as Cholera SMART II (New Horizons, Columbia, MD, USA) or the vertical flow immunochromatographic Crystal VC® RDT (Span Diagnostics, Surat, India) system (Dick et al., 2012; Page et al., 2012).

3.1. The changing focus of CIDT

While CIDT has already been with us for decades in one form or another, the underlying tenet for most of such testing in the past has been that it focused on identification of infectious agents that were either difficult or impossible to grow in vitro, were slow-growing and/or required extensive incubation periods (e.g., tuberculosis), or were so new or novel that not much information was available on their identification (*Tropheryma whipplei*). There are clearly exceptions to this rule. For instance, a number of different companies have in the past offered culture-independent testing in tandem for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* such as the Aptima Combo 2 panel by Hologic®/Gen-Probe (Gen-Probe Inc., San Diego, CA, USA). While the former agent is difficult to culture in vitro, the later is not. These systems, however, offer numerous advantages including higher sensitivity in males; they are cost effective (2 test results from 1 sample), allow use of urine specimens instead of urethral swabs, and have faster detection times (high volume test requests), which translates into a quicker medical and public health response for treatment and reduction in the prevalence and incidence of both diseases.

CIDT ground rules are now changing and no longer exist as originally defined previously. Recently commercially produced CIDT products are being manufactured that focus on different groups of infectious agents that are associated with common syndromes and that are not linked to sexual transmission. Systems, which have seen the greatest commercial development, involve molecular PCR-based platforms targeting syndromic infections such as acute gastroenteritis (AGE) (Gray and Coupland, 2014). These new systems differ in 2 fundamental ways from past CIDTs, namely, 1) they are aimed at detecting rapidly growing bacteria that are generally easy to isolate and 2) final identifications are achieved based upon a single non–culture-based technique. Table 1 lists several systems that are now commercially available on the international market. The oldest of these CIDTs for AGE are the EntericBio® System (Serosep, Limerick, Ireland) and the Seeplex® Diarrhea ACE system (Seegene, Korea) (Coupland et al., 2012; Koziel et al., 2013a, 2013b), which are used in

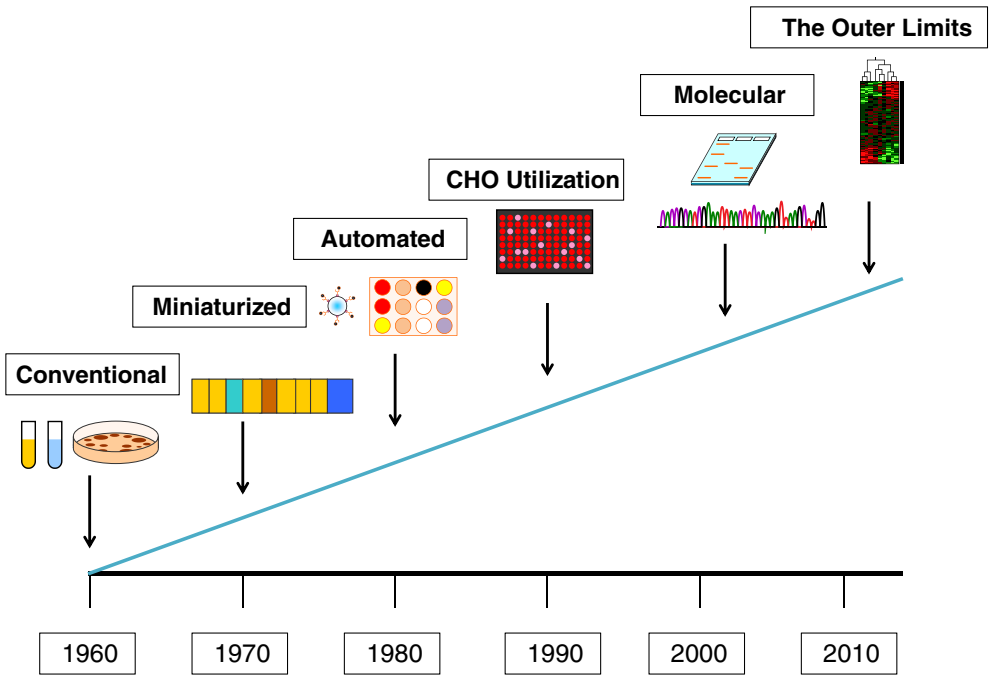

Fig. 1. Schematic chronologic transition of testing methodologies in the clinical microbiology laboratory, circa 1960–2010.