



Multiplex molecular diagnostic tests and the management of diarrhea: the wave of the future?

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Diarrhea is an extremely common clinical syndrome. Most cases are mild and self-limited. However, diarrhea remains the second leading cause of childhood death in resource-poor countries as well as the cause of significant morbidity [1]. A large and increasing number of pathogens have been identified, which can cause diarrhea and the optimal therapy varies with the pathogen. However, diagnosis of the causes of diarrheal disease has always been problematic.

Bacterial causes of diarrhea were identified in the 19th century and bacterial cultures became a standard approach to the identification of *Salmonella*, *Shigella*, and *Campylobacter* as well as cholera. However, bacterial cultures identify the causes of only a small proportion of acute diarrhea. In the mid-to-late 20th century, other organisms were identified as important causes, especially viruses such as rotavirus, norovirus, and adenovirus. Antigen-detection tests have been used for rotavirus, but most viral diarrhea has been assumed to not require specific therapy and thus has not been a major target for the development of diagnostics.

Studies in travelers and children in resource-limited countries identified an important role for diarrheagenic *Escherichia coli* in diarrhea. Indeed, many paradigms for management of traveler's diarrhea assume that *E coli* is the culprit. Nevertheless, diagnostic tests to identify diarrheagenic *E coli* have also been slow to develop and are still not available in most clinical settings. Instead, empiric antibiotics are a key part of recommendations for travelers and infants in resource-poor countries.

Protozoan parasites also play a role in diarrhea. *Entamoeba histolytica* was long recognized as a cause of dysentery; *Giardia* was associated with diarrhea and steatorrhea, whereas *Cryptosporidium* was associated with waterborne diarrhea in immunocompetent individuals. Diagnosis was largely based on microscopy. However, this has never been very sensitive. As each organism or group of organisms requires different testing, identification of causes has required multiple tests (bacterial cultures, viral antigens or PCR, stains and microscopy for parasites), which is expensive, time-consuming for

technical staff, and slow. Even then results are often delayed until after disease resolution. Furthermore, the cause of most cases remained unknown even with multiple tests.

Management guidelines for acute diarrhea in wealthy countries have focused on limiting the use of diagnostic tests to those at highest risk for abnormal results [2–4]. However, these paradigms often lack the sensitivity to identify treatable pathogens, such as *Shigella* [5]. By contrast, travelers and young children in resource-limited countries are treated empirically. However, that approach is now recognized to risk colonization with drug-resistant organisms and potential adverse consequences on the normal microbiome [6].

Advances in molecular diagnostics have begun to change the perception on the value of diagnostics. Now pathogens can be detected in the vast majority of cases of diarrhea [7,8]. Recent multicenter studies of diarrheal diseases in resource-poor countries are identifying diarrhea pathogens in nearly all subjects. Now another problem has emerged: most nondiarrheal specimens also contain one or more pathogen [9]. Thus, the presence of a pathogen is not an adequate proof of causation. In research studies, the problem of over-diagnosis can be overcome by including controls without diarrhea to establish background prevalence [7,8]. Thus, diarrhea is attributed only when the frequency of detection is higher than in the healthy population. This approach, however, may mask contributions for some pathogens, [10] particularly those that are shed for long periods of time after an acute diarrheal episode. Another approach to the obstacle

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of over-diagnosis has been using quantitative thresholds. Multiplex PCR has improved the ability to detect organisms. Now simpler methods such as film and bead arrays have led to commercially available multiplex assays that are exquisitely sensitive at detecting pathogens, previously only identified in research laboratories. For example, in a beta test of film array in our laboratory in Galveston, we noted several cases of diarrheagenic *E coli*, that could have responded to antibiotics therapy when treated symptomatically as well as viral illnesses that were treated with antibiotics.

Although multiplex molecular diagnostics are increasingly available, they remain quite expensive. Indeed, costs for diagnostic testing may outweigh the entire cost of care for a diarrheal episode. How should a clinician approach this? We believe that multiplex molecular techniques are essential for the accurate diagnosis of the causes of diarrhea. However, the cost of testing may limit the cost-effectiveness of this approach in self-limited episodes. Indeed, the cost may preclude testing in outpatients with mild, watery diarrhea, which can generally be managed with oral rehydration alone or with antimotility agents. By contrast, the value of a specific diagnosis likely outweighs the expense for hospitalized patients in wealthy countries.

Is there still a role for bacterial culture and antigen-detection as diagnostic approaches? Clearly, culture has the added value of isolating organisms for drug-sensitivity testing. However, this could be performed reflexively on positive results. Thus, it is probably time for laboratories to develop a 21st century approach, where molecular tests are the initial diagnostic method. Although this approach may be laudable, it is not yet feasible in resource-limited settings. However, even then, research studies that identify pathogens should employ the best diagnostic tests available to define the pathogens that are important in specific settings so that appropriate empiric treatment protocols can be

developed for different age groups and categories of patients, such as travelers or the immunocompromised. In addition, there is a clear and desperate need for the development of low-cost diagnostic tests with performance characteristics similar to multiplex molecular tests, because of the huge disease burden in resource-limited settings.

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