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A Rapid Latex Agglutination Test to Distinguish *Shigella* from *Escherichia coli*: Patching the MALDI-TOF "Achilles' Heel"

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ABSTRACT

Objectives: While MALDI-TOF has dramatically improved the quality and turn-around-times for organism identifications from culture, it is unable to differentiate the protein profiles of *Shigella* from closely-related *E. coli* which has created new problems. This study evaluated the Wellcolex Colour *Shigella* kit (Oxoid), a simple 2-minute/2-pool latex agglutination test, as a means to distinguish *Shigella* from non-lactose fermenting (NLF) oxidase-negative Gram-negative bacilli identified as *E. coli* by the VITEK MS PLUS system (bioMérieux; IVD database). The kit comprises two reagent pools, each of which contains a mixture of red and blue coloured latex particles with each latex type specifically sensitized to react with one (of four) species within the genus *Shigella*, but not with *E. coli*.

Methods: 238 isolates were used for this study: 114 retrospective strains comprising 87 *Shigella* (50 *S. flexneri*, 29 *S. sonnei*, 7 *S. boydii*, and 1 *S. dysenteriae*); 27 NLF *E. coli* derived from 4 large centralized clinical laboratories serving numerous health facilities in the Greater Toronto Area; and 124 semi-consecutive prospective NLF isolates identified from diverse specimen types on MacConkey agar as *E. coli* by *VITEK MS PLUS* at one laboratory site. To prevent bias, isolate identities were blinded during testing. The latex agglutination test was completed and interpreted as per kit instructions. Statistics were calculated using www.graphpad.com.

Results: All 87 *Shigella* reacted with the Wellcolex Colour *Shigella* kit (Sensitivity: 100%; 95%CI: 94.9-100) and all were identified correctly to the species level based on the reagent pool and colour of the latex agglutination following kit instructions. 149 of 151 NLF *E. coli* correctly did not agglutinate with either latex pool in the kit (Specificity: 98.7%; 95%CI: 95.0-100). The 2 NLF *E. coli* that cross-reacted reproducibly did so with Reagent-1 blue latex which would be interpreted as *S. flexneri* based on kit instructions. Both were confirmed to be *E. coli* (VITEK 2 GNI, API 20E, no reactivity with the Remel *Shigella* anti-sera) and on retesting from 48h cultures, the reactions became clearly recognizable as auto-agglutination (clumping). The resulting specificities (95%CI) by species type for *S. flexneri*, *S. sonnei, S. boydii, and S. dysenteriae*-sensitized latex particles were 98.9% (96-99.96), 100% (97.8-100), 100% (98-100), and 100% (98.1-100), respectively.

Conclusions: This study demonstrates that the Wellcolex Colour *Shigella* kit is able to accurately and rapidly distinguish *Shigella* from *E. coli* in NLF, oxidase-negative Gram-negative bacilli identified as *E. coli* by *VITEK MS PLUS*. It is a low-complexity, cost-effective solution that provides results in a few minutes without disrupting workflow. A negative Wellcolex Colour *Shigella* kit result rules out *Shigella* spp. without the need for additional testing. However, since 2 *E. coli* were shown to cross-react with the *S. flexneri* sensitized latex, positive results should always be confirmed by an alternate means.

INTRODUCTION & OBJECTIVES

While MALDI-TOF mass spectrometry (MS) revolutionized organism identification in the microbiology laboratory, it also introduced new challenges. One such challenge is that the protein spectra of *E. coli* and *Shigella* spp. are indistinguishable. This shortcoming has preoccupied MS users globally. Many work-around options have been proposed: some entail over-night tests which delay reporting, or are laborious and disrupt workflow; for others, the identification accuracy may be sub-optimal.

To understand the specimen distribution of *Shigella* isolates, 5 centralized Toronto laboratories performed retrospective searches, where possible, back to 2000. This elucidated that most non-stool *Shigella* were from blood and that no single isolate had been identified from urine in >12 yrs.

From stool, non-lactose-fermenting (NLF) *E. coli* by MALDI-TOF would automatically be viewed as possible *Shigella*. But it is from blood, where *E. coli* is the predominant isolate, that a MALDI-TOF misidentification of *Shigella* as *E. coli* is most likely to go unnoticed. However, *Shigella* are typically NLF and *E. coli* are mostly lactose-fermenters, thus it is only the NLF from non-urine clinical specimens that MALDI-TOF identify as *E. coli*, that require further testing to rule out *Shigella*.

This study aimed to identify a pragmatic approach to resolve this issue. An acceptable test would have to be: simple (not impede workflow), rapid (not delay reporting), highly accurate and reproducible, and cost effective. To this end, the Remel Wellcolex Colour *Shigella* latex agglutination kit (Oxoid) was evaluated as a possible solution for distinguishing *Shigella* from NLF isolates identified by MALDI-TOF as *E. coli.*

MATERIALS & METHODS

The Remel Wellcolex Colour *Shigella* kit (Oxoid) is a CE-approved latex agglutination kit that claims to detect a diversity of *Shigella* serotypes, variants and forms (Table 1 below).

Table 1. Species subtypes detected by the Remel Wellcolex Colour *Shigella* Kit from Oxoid

Shigella boydii	**serotypes 1-15 (not 16-18 > rare)**
Shigella dysenteriae	**serotypes 1-12**
Shigella flexneri	**serotypes 1-6 X/Y variants**
Shigella sonnei	**forms I + II**

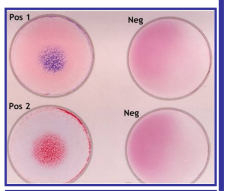

Figure 1. Positive and negative Wellcolex Colour *Shigella* latex agglutination reactions

The kit comprises 2 latex test reagents, each of which contains a mixture of blue and red sensitized particles. Reagent 1 detects *S. flexneri* and *S. sonnei* while Reagent 2 detects *S. boydii* and *S. dysenteriae*. For each reagent, as shown in Figure 1 using latex controls, positive reactions appear as BLUE agglutination with a red background (Pos 1), or as RED agglutination with a blue background (Pos 2). In the absence of agglutination, negative (Neg) pools are purple due to the combined unbound particles in solution.

Test procedure as per insert: pick a few colonies using toothpick (supplied); mix in 0.5mL saline (supplied) until smooth suspension; add single drops to 2 wells on test card using transfer pipette (supplied); add drop of Reagent 1 and 2 to top and bottom wells, respectively; mix pools and rotate card on flat-bed shaker; after 2 min, observe pools for RED, BLUE or NO agglutination.

Interpretation was as per insert. If agglutination was present in Reagent 1, BLUE indicated *S. flexneri* and RED indicted *S. sonnei,* while for Reagent 2, BLUE indicated *S. boydii* and RED indicted *S. dysenteriae*. If neither 1 nor 2 reagents agglutinated, the test isolate was documented as not *Shigella* spp.

While agglutination may be done from any agar (5% Sheep Blood, Hektoen, chromogenic agars, etc), this study used colonies grown on MacConkey agar since NLF *E. coli* by MALDI-TOF was to be established as criteria for testing.

A total of 114 retrospective isolates from 5 Toronto laboratory archives and ATCC control strain collections were tested. These comprised 87 *Shigella* spp. (52 *S. flexneri*, 29 *S. sonnei*, 7 *S. boydii*, 1 *S. dysenteriae*) and 27 NLF *E. coli*.

Similarly, a total of 236 prospective semi-consecutive NLF isolates identified at MSH/UHN by VITEK MS PLUS (bioMérieux) as *E. coli* were tested. These comprised 235 *E. coli* and 1 *S. sonnei* from blood, urine, stool or ESBL screens.

All retrospective *Shigella* and the prospective stool isolate (*S. sonnei*) had confirmed species level identities from the Ontario Public Health laboratory, (OPHL) while *E. coli* were confirmed using the VITEK 2 GNI card (bioMérieux).

The identities of the total 350 study isolates were blinded prior to testing with the Remel Wellcolex *Shigella* kit, and latex agglutinations were carried out in accordance with manufacturers' instructions.

The sensitivities and specificities for detection of each *Shigella* species independently were determined, and 95% confidence intervals were calculated using the on-line QuickCalcs program www.graphpad.com.

RESULTS

The sensitivities for detection each of the 4 *Shigella* spp. and specificities with respective 95% confidence intervals obtained for the Wellcolex Colour *Shigella* kit are presented in Table 2. Agglutination reactions for the 350 strains were compared to negative and positive control latex antigen to the to ensure all kit reagents were in control.

Agglutination results from the 88 *Shigella* spp. were as follows:
- 52 of 52 (100%) *S. flexneri* agglutinated BLUE in Reagent 1 (NEG in 2)
- 30 of 30 (100%) *S. sonnei* agglutinated RED in Reagent 1 (NEG in 2)
- 7 of 7 *S. boydii* agglutinated BLUE in Reagent 2 (NEG in 1)
- 1 of 1 *S. dysenteriae* agglutinated RED in Reagent 2 and (NEG in 1)

Similarly, agglutination results from the 262 *E. coli* were as follows:
- 260 of 262 (99.2%) No agglutination = Not *Shigella* spp.
- 2 (0.8%) isolates agglutinated BLUE in Reagent 1, suggesting a possible *S. flexneri.* However, VITEK 2 GNI and API biochemicals indicated *E. coli*, and Wellcome *Shigella* type anti-sera were negative for both. As retesting again found agglutination in Reagent 1, even though it was noted that the BLUE clumping was "stringy" from older colonies , isolates were sent the Ontario Public Health Laboratory for further testing and *Shigella* spp. was ruled out for both isolates. These were considered to be reproducible false-positives of the Remel Wellcolex Colour *Shigella* kit.

Table 2. Remel Wellcolex Colour *Shigella* kit performance obtained from blinded testing of 350 NLF isolates comprising 88 *Shigella and* 262 *E. coli*

Study Outcomes	For *S. flexneri*	For *S. sonnei*	For *S. boydii*	For *S. dysenteriae*
Sensitivities 95% CI	100.0% 91.8-100%	100% 86.5-100%	100% 59.6-100%	100% 50-100%
Specificities 95% CI	99.3% 97.4-99.98%	100% 98.6-100%	100% 98.7-100%	100% 98.7-100%

CONCLUSIONS

The Remel Wellcolex Colour *Shigella* kit was found to be:

- ✓ Highly accurate - sensitivities (100%), specificities (99.3-100%)
- ✓ Low-complexity test that was quick and easy to use
- ✓ Easily integrated into existing workflow with no reporting delay
- ✓ Cost-effective

When to rule out *Shigella?*

- ✓ NLF identified by MALDI-TOF MS as *E. coli* (LF are okay)
- ✓ Blood, enteric, respiratory, "miscellaneous" specimen isolates
- × Not urine or ESBL/carbapenemase surveillance isolates

All *Shigella* must continue to be confirmed at reference laboratories

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