Background

• While MALDI-TOF has revolutionized microbial identification in clinical laboratories, it has also introduced some new challenges.

• One such challenge is that MALDI-TOF spectra from *E. coli* and *Shigella* spp. are indistinguishable, regardless of toxin phenotype.

• So for VITEK MS users, bioMérieux has rightly placed a disclaimer right on the REVIEW SCREEN to ensure that everyone understands that any *E. coli* may in fact be a *Shigella* spp.

• This issue has pre-occupied many MALDI-TOF users globally, and consequently, a wide range of possible work-around options have been suggested, but most are laborious or require over-night conventional testing, the ID of which may also be sub-optimal.
So, what do we know?

- *Shigella* spp are uniformly non-lactose fermenters (NLF) on MacConkey agar
- *E. coli* are predominantly LF on MacConkey, but many circulating strains are NLF (including ESBL)
- From urines, a retrospective search for *Shigella* at MSH/UHN identified zero *Shigella* isolates since 2000 (14 hospitals over 12.5yrs)
- Over the same period, most non-stool clinical *Shigella* were isolated from blood cultures
- Since *E. coli* is the most common Gram-negative bacillus identified from blood, it is at this site that *Shigella* identified as *E. coli* by MALDI-TOF is most likely to occur
- From stool, where *Shigella* are anticipated, all *E. coli* by MALDI-TOF would naturally be viewed as potential *Shigella*

Objectives

- To formulate a simple pragmatic approach to ensure that *Shigella* isolates from blood cultures are not misidentified
  - Must minimize confirmatory work and must eliminate reporting delays
- To streamline implementation of VITEK MS onto the urine bench
  - Reduce isolate types requiring MALDI-TOF testing
- To this end, studies were undertaken to validate potential solutions for distinguishing *E. coli* from *Shigella* spp.:
  - Determine specificity of chromogens of urine agars for *E. coli* vs. *Shigella* spp.
  - Validation of a *Shigella* latex kit for use as a simple, rapid confirmatory test
- Finally, to present data in support of possible workflow algorithms that utilize VITEK MS, chromogenic agar and latex agglutination to resolve the *Shigella/E. coli* MALDI-TOF ID ambiguities and to enhance VITEK MS workflow for clinical laboratories
### Evaluation studies 2013

• 3 urine chromogenic agars, including the bioMérieux chromID CPS ID 4, were evaluated to determine the most reliable medium for distinguishing *E. coli* from *Shigella* spp.
  - Prospective testing included 2500 urines
  - Retrospective testing of 455 isolates of 45 distinct species including 86 well-characterized *Shigella* spp.

• Validation of a simple latex agglutination test for rapid detection and *Shigella* species identification, and for use in eliminating *E. coli*
  - Wellcolex Colour Shigella
  - Retrospective testing of 86 well-characterized *Shigella* and 28 genetically diverse NLF *E. coli* clinical isolates
  - Prospective testing of 42 NLF *E. coli* clinical isolates

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### chromID CPS ID 4 agar

**Prospective evaluation - URINE**

<table>
<thead>
<tr>
<th>2500 WASP plated urines (1 µL)</th>
<th>MacConkey/5% Sheep Blood Agar = MSH protocol</th>
<th>5 lots (500 ea): chromID CPS4, Agars “B” and “C”</th>
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<tbody>
<tr>
<td>222 (8.9%) urines grew significant <em>E. coli</em></td>
<td>180 (81.1%) LF, 42 (19.9%) NLF on MacConkey</td>
<td>212 (100%) Burgundy pink on chromID CPS 4</td>
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<td></td>
<td>219 (98.7%) Burgundy pink on “B”</td>
<td>207 (94.1%) Burgundy pink on “C”</td>
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<td>0/2278 (0%) Non-<em>E. coli</em> were Burgundy pink on any agar</td>
</tr>
</tbody>
</table>

- Predictability of *E. coli* growing Burgundy pink on chromID CPS4 agar:
  - Sensitivity 100% (95%CI: 98-100)
  - Specificity 100% (95%CI: 99.8-100)

- Although there were no *Shigella* in the study,
  - 42 NLF *E. coli* by VITEK MS needed confirmatory ID to rule out *Shigella* vs. NONE from chromID CPS4
  - CPS4 P<0.0001 less work to ID UTI *E. coli*
Plated to each of 5 lots of chromID CPS4 were:
- btw 163-369 non-Shigella = 1497 tests (Incl. 45 species)
- btw 26-86 distinct Shigella = 370 tests
  - 50 flexneri, 28 sonnei, 7 boydii, 1 dysenteriae
- Overall retrospective challenge: 1867 tests

- Of 1497 non-Shigella tests, 106 (7%) were E. coli (btw 17-23/lot)
  - 102/106 (96.2%) grew Burgundy pink
  - 4 (3.8%) grew Light pink (1 NLF E. coli on 4/4 lots)
- Of 1391 non-Shigella/non E. coli, 1 (0.07%) grew Burgundy pink
- Species ID TO BE CONFIRMED (entclo)

- Of 370 Shigella tests, 102 (27.6%; 25/28 S. sonnei) grew Burgundy pink
  - 3/28 S. sonnei + 263 tests/58 Shigella grew clear cols (PHL conf ID

When simulating blood where Shigella spp. may be misidentified by VITEK MS, chromID CPS4 was unable to distinguish S. sonnei from E. coli
>> confirmatory testing of NLF “E. coli” from MacConkey agar is required!

Wellcolex Colour Shigella Test

- Simple 2 minute CE-approved test
- Inoculated using colonies from diverse agars:
  * MacConkey, 5% Sheep Blood, Hektoen chromID CPS ID 4, etc
- Touch few colonies with supplied toothpick
- Mix into 0.5mL saline in supplied tube
- Transfer drop onto 2 spots on supplied card
- Add Reagent 1 to top well, mix
- Add Reagent 2 to bottom well, mix
- Rotate on flat-bed shaker for 2 minutes
- Observe for red or blue agglutination

Compare to control latex
- Blue in Reagent 1 = S. flexneri
- Blue in Reagent 2 = S. boydii
- Red in Reagent 1 = S. sonnei
- Red in Reagent 2 = S. dysenteriae
- No agglutination = not Shigella spp.

Made by Remel Europe
Available from Oxoid
Wellcolex Colour Shigella Results

- 85 confirmed Shigella from blood or stool
  - From 4 major GTA/Peel Region labs-10 years
- 70 NLF E. coli from 2 major GTA laboratories
- 4 Shigella and 2 E. coli ATCC control strains
- 161 isolates tested blinded to species-ID
  - From MacConkey or 5% sheep blood agars

Compared to control latex:
- Blue+ Reagent 1 = 52/52 S. flexneri
- Red+ Reagent 1 = 29 /29 S. sonnei
- Blue+ Reagent 2 = 6/7 S. boydii
  - 1 S. boydii auto-agglutinated from old plate
  - Rpt test from fresh sub > Blue in Reagent 2
- Red+ Reagent 2 = 1/1 S. dysenteriae
- 72 with No agglutination = Not Shigella spp.
  - 70 NLF E. coli plus 2 LF E. coli controls
- Sensitivity: 89/89 = 100% (95% CI: 95-100)
- Specificity: 72/72 =100% (95% CI: 93.9-100)

Overall conclusions

- From this interim analysis, when VITEK MS is implemented on the urine bench, the use of chromID CPS4 would be a distinct advantage to improve the workflow since **burgundy pink** growth was 100% sensitive and >99.98% specific for E. coli

- However, from the blood bench, isolates that are NLF on MacConkey and E. coli by VITEK MS should be confirmed to rule out Shigella spp.
  - The Wellcolex Colour Shigella Latex agglutination kit was found to be a useful rapid test to achieve this goal