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Evaluation of Molecular Methods for Identification of *Salmonella* Serovars

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Classification by serotyping is the essential first step in the characterization of *Salmonella* isolates and is important for surveillance, source tracking, and outbreak detection. To improve detection and reduce the burden of salmonellosis, several rapid and high-throughput molecular *Salmonella* serotyping methods have been developed.

The aim of this study was to compare three commercial kits, Salm SeroGen (Salm Sero-Genotyping AS-1 kit), Check&Trace (Check-Points), and xMAP (xMAP *Salmonella* serotyping assay), to the *Salmonella* genoserotyping array (SGSA) developed by our laboratory. They were assessed using a panel of 321 isolates that represent commonly reported serovars from human and nonhuman sources globally. The four methods correctly identified 73.8% to 94.7% of the isolates tested. The methods correctly identified 85% and 98% of the clinically important *Salmonella* serovars Enteritidis and Typhimurium, respectively. The methods correctly identified 75% to 100% of the nontyphoidal, broad host range *Salmonella* serovars, including Heidelberg, Hadar, Infantis, Kentucky, Montevideo, Newport, and Virchow. The sensitivity and specificity of *Salmonella* serovars Typhimurium and Enteritidis ranged from 85% to 100% and 99% to 100%, respectively.

It is anticipated that whole-genome sequencing will replace serotyping in public health laboratories in the future. However, at present, it is approximately three times more expensive than molecular methods. Until consistent standards and methodologies are deployed for whole-genome sequencing, data analysis and interlaboratory comparability remain a challenge. The use of molecular serotyping will provide a valuable high-throughput alternative to traditional serotyping. This comprehensive analysis provides a detailed comparison of commercial kits available for the molecular serotyping of *Salmonella*.

Food-borne illness is associated with significant human and societal costs worldwide. *Salmonella* is one of the most common causes of gastrointestinal infection and results in tens of millions of human infections globally each year (http://www.who.int/mediacentre/factsheets/fs139/en/). Since not all cases are tested or reported, this number is estimated to be as high as 1.3 billion (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/salmonella-ent-eng.php). In Canada, *Salmonella* is the most prevalent food-borne illness to cause bacteria. It is estimated that, of the 4 million cases of food-borne illnesses that occur annually, nontyphoidal *Salmonella* accounts for approximately 41% of these reported infections in Canada (1). More than 100,000 cases of salmonellosis are reported annually in the European Union (EU) (http://www.efsa.europa.eu/en/topics/topic/salmonella). In humans, these infections can range from acute in healthy individuals to serious systemic disease in immunosuppressed individuals. Typically, the source of infection is contaminated food or water (2). The annual cost of illness due to food-borne pathogens is substantial. In the United States alone, *Salmonella* is estimated to cause an annual loss of $3.3 billion U.S. dollars (USD) due to destruction of contaminated food sources, loss of work productivity, and health-care costs (3). The estimated cost of human salmonellosis in the EU is €3 billion a year (http://www.efsa.europa.eu/en/topics/topic/salmonella). Because *Salmonella* has such global significance, the estimated global economic burden is inconceivable.

Food-borne illness and outbreaks also place a global burden on health-care systems. In Canada, cases and outbreaks must be investigated to secure public health, and resources such as laboratory testing and epidemiological analyses are timely and expensive (4). The burden placed on Canada and other countries can be alleviated by rapidly detecting salmonellosis to limit its spread and impact.

Salmonella is a Gram-negative bacterium comprising two species, *S. enterica* and *S. bongori*, which encompass more than 2,600 serovars that are capable of causing infection in a wide range of hosts (5). Due to the limited sequence variability in many prevalent serovars, subtyping is required for accurate identification and outbreak investigation. Since the 1930s, *Salmonella* serovars have been identified by serotyping using the White-Kauffmann-Le Minor (WKL) scheme to determine phenotypic antigens, resulting in a massive library of information and data. The molecular typing method of pulsed-field gel electrophoresis (PFGE) has been used since the 1990s and is currently considered the gold standard for further discrimination and analysis (6). Although serotyping and

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PFGE have held their place in the reference laboratories for decades, there are drawbacks to each typing method. Both methods are time-consuming and require expensive reagents, equipment, and highly trained staff (6, 7). However, due to the wealth of information collected from traditional serotyping and PFGE, it is important that new high-throughput molecular methods produce data comparable to historical data.

Rapid molecular detection of *Salmonella* is paramount to public health (8). DNA-based molecular techniques allow for the detection and characterization of isolates at the genetic level, while traditional techniques, such as serotyping, detect phenotypic properties that may not always be expressed, regardless of the presence of the genetic material. Other advantages are that molecular typing is faster, does not require the same technical expertise as nonmolecular techniques, has high-throughput capabilities, and can generate a wealth of objective information in a relatively short amount of time (6). Although the future of *Salmonella* identification and characterization will rely on whole-genome sequencing (WGS) as complementary to or replacing traditional serotyping, it is not ready for routine use in most diagnostic laboratories. WGS remains more expensive than molecular methods, with data analysis and interlaboratory comparability of WGS data still posing a challenge for most public health laboratories. Until these WGS issues are resolved, molecular serotyping will provide a high-throughput alternative to traditional serotyping. There are several molecular techniques currently available to laboratories for the rapid molecular identification of *Salmonella* serotype.

The aim of this study is to compare molecular *Salmonella* serotyping technologies in terms of ease of use, cost, and reliability of results, with the most commonly reported serovars from human and nonhuman sources globally. This study evaluated three commercially available molecular methods for the identification of *Salmonella* serovars, including *Salmonella* serogenotyping assay (Alere Technologies; 9), hereafter called Salm SeroGen, Check&Trace *Salmonella* (Check-Points; 10), and xMAP *Salmonella* serotyping assay (Luminex; 11), and the *Salmonella* genoserotyping array (SGSA; 7, 12) developed by our laboratory. All of the molecular methods have been validated and are accredited by ISO or the World Organisation for Animal Health (OIE). This study evaluated four methods using the same panel of *Salmonella enterica* isolates representing 143 serovars.

To our knowledge, this is the first study to comprehensively compare all commercially available molecular methods for *Salmonella* serotyping using the same panel of isolates. Beaubrun et al. (13) compared Check&Trace, xMAP, and multiplex PCR. Other noncommercial methods have been proposed (14) but were not evaluated in this study.

MATERIALS AND METHODS

Bacterial strains and culture methods. The panel of 321 isolates was selected to represent the most globally prevalent serovars from human and nonhuman sources, many of which were previously used in a validation study (12). For the common serovars Typhimurium and Enteritidis, 20 isolates of each were tested. For the prevalent and/or broad host range serovars (Hadar, Heidelberg, Infantis, Kentucky, Montevideo, Newport, and Virchow), seven isolates of each were tested. For the remaining serovars expected to be detected by the methods, three isolates of each were tested. In addition, the panel included serovars for which the molecular methods were not expected to detect but provided coverage of almost all of the antigens in the WKL scheme, to assess specificity. One isolate of each of these serovars was tested. All isolates were traditionally serotyped in a reference laboratory at the National Microbiology Laboratory in Guelph, Canada, and were from human, animal, and environmental sources. *Salmonella* strains were grown overnight at 37°C on Luria-Bertani agar (BD Canada, Mississauga, ON, Canada). The antigenic formula of each strain was determined using standard methods (15, 16), and the serovar was assigned according to the WKL scheme in an OIE/ISO accredited laboratory (5).

TABLE 1 Performance of four molecular *Salmonella* serotyping methods

Method	No. of isolates tested	No. of serovars tested	No. of isolates correct	No. of serovars correct[a]	% isolates correct	% serovars correct[a]
SGSA	321	143	304	136	94.7	95.1
Salm SeroGen	321	143	237	80	73.8	55.9
Check&Trace	321	143	266	109	82.9	76.2
xMAP	321	143	284	120	88.5	83.9

[a] 100% correct for each isolate tested per serovar.

***Salmonella* genoserotyping array.** The SGSA microarray and procedures were as described previously (7), with some modifications as outlined by Yoshida et al. (12).

***Salmonella* serogenotyping assay (Salm SeroGen).** DNA prepared for the SGSA panel was also used in running the Salm SeroGen panel. Briefly, the differences from the Salm SeroGen kit recommendations are as follows. Luria-Bertani agar (BD Canada) was used to grow bacterial strains instead of the recommended kit instructions to use 2× tryptone yeast agar, and the EZ1 DNA tissue kit and BioRobot (Qiagen Ltd., Mississauga, ON, Canada) were used to extract DNA instead of the DNeasy blood and tissue kit (Qiagen). If the DNA gave a negative result, DNA was re-extracted using the EZ1 DNA kit with the addition of 4 μl of RNase (Qiagen) to each sample and incubated at room temperature for 2 min, and then the sample was run on the BioRobot.

All DNA was quantified spectrophotometrically using the NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE) to determine the concentration and volume required in the assay and then diluted with molecular-grade water prior to being used, if required (*Salmonella* Serogenotyping Assay User Guide 13-02-15-001-V1).

Check&Trace *Salmonella*. Check&Trace was processed according to the manufacturer's recommendations (Check&Trace *Salmonella* User Manual, version 9.1, issued 16 December 2011).

xMAP *Salmonella* serotyping assay. xMAP was processed according to the manufacturer's recommendations (xMAP part no. 89-60000-00-091 Revision A, June 2012), with the exception of the DNA extraction protocol, in which a 1-μl loopful of DNA was extracted using 20 μl of InstaGene Matrix (Bio-Rad, Mississauga, ON, Canada) at 56°C for 30 min at 550 rpm and then at 95°C for 8 min at 550 rpm (Instagene matrix insert, LIT544 Revision G).

Interpretation of results. Isolates were considered correctly identified if the result reported by the molecular method was the same serovar as determined by traditional serotyping, and nontarget serovars were considered correct if not misidentified as a target serovar. Target serovars are defined as serovars that can be identified by each of the kits, as listed in the respective product insert. Nontarget serovars are defined as serovars not listed by the respective product insert and are used for evaluation of specificity. Interpretation and scoring of SGSA results was the same as described by Yoshida et al. (12) for the 57 target serovars.

Statistical methods. Test sensitivity and specificity of each method were evaluated relative to traditional serotyping by 2-by-2 table analysis (17) for *Salmonella* serovars Enteritidis and Typhimurium. Specificity = TN/(TN + FP) and Sensitivity = TP/(TP + FN), where TN is true negative, TP is true positive, FP is false positive, and FN is false negative.

TABLE 2 Accuracy of detection of common *Salmonella* serovars using four molecular methods

Serovar	Method	Total no. of isolates tested	No. of isolates correctly identified	No. of technical repeats	% Correct
Enteritidis	SGSA	20	17		85.0
	Salm SeroGen		19		95.0
	Check&Trace		17	6	85.0
	xMAP		20	2	100.0
Typhimurium (incl. var. Copenhagen and I:1,4,[5],12:i:-)	SGSA	20	20	1	100.0
	Salm SeroGen		20	4	100.0
	Check&Trace		19		95.0
	xMAP		19	2	95.0
Hadar	SGSA	7	7	1	100.0
	Salm SeroGen		7	1	100.0
	Check&Trace		7		100.0
	xMAP		7		100.0
Heidelberg	SGSA	7	7		100.0
	Salm SeroGen		7	1	100.0
	Check&Trace		7	1	100.0
	xMAP		6	4	85.7
Infantis	SGSA	7	7		100.0
	Salm SeroGen		7		100.0
	Check&Trace		7		100.0
	xMAP		7		100.0
Kentucky	SGSA	7	7		100.0
	Salm SeroGen		7	2	100.0
	Check&Trace		0		0.0
	xMAP		7		100.0
Montevideo	SGSA	7	6	3	85.7
	Salm SeroGen		7		100.0
	Check&Trace		7	2	100.0
	xMAP		7	1	100.0
Newport	SGSA	7	7		100.0
	Salm SeroGen		7		100.0
	Check&Trace		7		100.0
	xMAP		7	2	100.0
Virchow	SGSA	7	7		100.0
	Salm SeroGen		7		100.0
	Check&Trace		5	1	71.4
	xMAP		7	3	100.0

RESULTS

The performance of four molecular *Salmonella* serotyping methods was assessed, including the SGSA, Salm SeroGen, Check&Trace, and xMAP. The latter three are commercially available. The four methods correctly identified 73.8% to 94.7% of 321 isolates tested. Of the 143 serovars tested, 55.9% to 95.1% were correctly identified (Table 1). The four methods correctly identified 85% and 98% of the prevalent and clinically important *Salmonella* serovars Enteritidis and Typhimurium, respectively (Table 2). The methods correctly identified 75% to 100% of the nontyphoidal, broad host range *Salmonella* serovars, including Heidelberg, Hadar, Infantis, Kentucky, Montevideo, Newport, and Virchow (Table 2). *Salmonella* serovars Hadar, Infantis, and Newport were identified correctly every time they were tested by each method. Check&Trace was unable to discriminate the seven *Salmonella* serovar Kentucky samples more completely than *Salmonella* genovar (Table S1 in the supplemental material), although serovar Kentucky is on the list of AOAC Research Institute-certified serovars identified by this method.

Twenty of each of the *Salmonella* serovars Typhimurium and Enteritidis were tested by each method. xMAP was able to discriminate *Salmonella* serovar Typhimurium var. 5 (var. Copenhagen) from serovar Typhimurium. Two of the five monophasic strains of serovar Typhimurium were consistently identified by all four methods as antigenic formula I:1,4,[5],12:i:-, while the remaining three were identified as serovar Typhimurium because they were positive for the presence of *fljB*, the gene that encodes the phase 2 flagellar antigen. Check&Trace and xMAP were able to identify *Salmonella*

TABLE 3 Sensitivity and specificity of *Salmonella* serovars Enteritidis and Typhimurium

	Method	True positive	True negative	False positive	False negative	Total no. tested	Sensitivity	Specificity
Salmonella	SGSA	17	301	1	3	321	85.0	99.7
serovar	Salm SeroGen	19	301	8	1	321	95.0	97.4
Enteritidis	Check&Trace	17	301	2	3	321	85.0	99.3
	xMAP	20	301	3	0	321	100.0	99.0
Salmonella	SGSA	20	301	0	0	321	100.0	100.0
serovar	Salm SeroGen	20	301	1	0	321	100.0	99.7
Typhimurium	Check&Trace	19	301	0	1	321	95.0	100.0
	xMAP	19	301	0	1	321	95.0	100.0

serovar Enteritidis, while the SGSA and Salm SeroGen were unable to distinguish it from the closely related serovars Blegdam (D:g,m,q:-), Moscow (D:g,q:-), and Nitra (D:g,m:-). Test sensitivity and specificity was calculated for the *Salmonella* serovars Enteritidis and Typhimurium for each assay (Table 3). Sensitivity ranged from 85% to 100%, and specificity ranged from 99% to 100%.

The type of incorrect serovar identification varied for each assay, from identifying a serovar with a similar antigenic formula to incomplete identification. Samples uniquely misidentified by each method, including incomplete identification, are described in Table S1 in the supplemental material.

Five of the seven SGSA misidentifications were due to incorrect H antigen gene identification within the g-complex, which has high sequence similarity. Two misidentifications were due to incorrect H antigen gene identification within the l-complex, which also share high sequence similarity. The H antigen gene discrimination resulted in four isolates being misidentified as serovars with similar antigenic formulae, and three isolates were incompletely identified.

Of the 48 uniquely misidentified by Salm SeroGen, 42 had a score greater than 6.5, meaning that the confidence in the result was low. During this study, we frequently obtained a score above 6.5 for Salm SeroGen test results. It is recommended in the product insert that a score above 6.5 excludes reliable strain identification, although a serovar result is provided. However, if a maximum score of 6.5 was used in this study, results would not have been obtained for 53% (170 of 321) of the samples tested, whereas only 38% (64 of 170) of these samples were incorrectly identified when ignoring scores above 6.5. In 11 of the 321 samples tested by Salm SeroGen, one of the three *Salmonella* controls was negative. The product insert highlights that *inv*A, *gal*F, and *man*C confirm the presence of *Salmonella*, but it is unclear how to treat a sample when one or more of these controls are negative. Many of the 48 isolates misidentified by this method were not common serovars. Twelve isolates were misidentified as serovars with similar antigenic formulae, and 36 isolates were misidentified as unrelated serovars.

The 24 misidentified samples by Check&Trace were mainly due to insufficient characterization, as 20 of the 24 were identified as "*Salmonella* genovar" and the serovar was not provided. Two isolates were misidentified as serovars with similar antigenic formulae, and two were misidentified as unrelated serovars.

Five of the seven xMAP misidentifications were within serogroup D. Two samples belonging to serogroup C1 were not detected; one was misidentified as serogroup B, resulting in Mbandaka (C1:z_{10}:e,n,z_{15}) being misidentified as Stanleyville (B:z_{10}:e,n,z_{15}), and the other did not identify a serogroup. Four isolates were incompletely identified, two isolates were misidentified as serovars with similar antigenic formulae, and one isolate was misidentified as an unrelated serovar.

Four non-*Salmonella* samples commonly found in clinical samples (*Escherichia*, *Campylobacter*, and *Enterobacter*) were also tested, and the *Salmonella* controls were negative for all four methods (data not shown). SGSA, Check&Trace, and xMAP reported as non-*Salmonella*, but despite *Salmonella* controls being negative, Salm SeroGen reported *Salmonella* serovars Minnesota, 66:z_{41}:-, and Enteritidis.

Repeat testing (second pass) was completed on samples with incomplete identification, those not matching a serovar in WKL, to account for technical errors and more accurately reflect the ability of each method (Table 4). The first pass resulted in a 10% error (5.9% to 21.2%) for the various methods. A second pass resolved 100% of technical errors and produced correct results.

There were two serogroup B samples that were misidentified by all four methods; serovar Borreze (54:f,g,s:-) was consistently misidentified as serovar Agona (B:f,g,s:[1,2]), and serovar Crossness (67:r:1,2) was consistently misidentified as serovar Heidelberg (B:r:1,2). Although these serovars are not listed by the methods, they were incorrect because they were misidentified as common serovars listed by all four methods. Repeat traditional serotyping confirmed the respective samples as serovars Borreze and Crossness.

DISCUSSION

Molecular serotyping methods offer a high-throughput alternative to traditional serotyping, which can strengthen the public health response capacity. In this study, we evaluated the performance of four molecular methods for *Salmonella* molecular serotyping. All of the methods assessed required the use of pure cultures for effective detection. Three of the four methods targeted somatic and flagellar genes, whereas the gene targets of Check&Trace are unpublished. Molecular methods are able to

TABLE 4 Summary of repeat testing

	SGSA	Salm SeroGen	Check&Trace	xMAP
No. of samples tested	321	321	321	321
No. correct 1st pass	285	215	237	216
% correct 1st pass	88.8	67	73.8	67.3
No. correct 2nd pass	304	237	266	284
% correct 2nd pass	94.7	73.8	82.9	88.5

detect genes that are not expressed and therefore not identified by traditional serotyping. The identification of additional information at the genetic level may be very useful in an epidemiological context. The four methods correctly identified 73.8% to 94.7% of the 321 isolates tested. The percentage of serovars identified correctly was slightly lower (55.9% to 95.1%) than the percentage of isolates identified correctly, as it required each isolate within a serovar to be correct for the serovar to be considered correct. There was a small amount of variation in the results among the strains belonging to the same serovar (Table 2). For example, three *Salmonella* serovar Enteritidis were identified as a serovar with a very similar antigenic formula by the SGSA, but these isolates were correctly identified by other methods. Two isolates of serovars Typhimurium and Heidelberg were misidentified by xMAP as monophasic, while they were correctly identified as diphasic by the other methods. The variation between methods may have been due to the performance of the individual probes. The variation within methods was due to incomplete identification when tested by Check&Trace and the misidentification of serovars with very similar antigenic formulae when tested by SGSA, indicating sequence similarity in the targeted genes. Hendriksen et al. (18) reported similar findings with traditional serotyping. During international ring trials, incorrect serovar identification was often caused by incorrect detection of the phase 2 flagellar antigen, in particular within the H antigen complexes (E, G, and L).

The methods evaluated in this study were based on either microarray or bead-based technology. The technology type did not have a significant impact on performance because the incorrect identification of serovars was not specific to one technology. The gene targets (probes) seem to have the most impact on the performance of a method, as the different technologies each misidentified a number of samples that other methods identified correctly. Overall, the SGSA and xMAP had the lowest number of misidentifications ($n = 7$ isolates), Check&Trace misidentified 24 isolates, and Salm SeroGen had the highest number of misidentifications ($n = 48$ isolates) (Table S1 in the supplemental material).

The average technical error for the four methods was 10% and was due to possible cross-contamination of samples or a technical error made during PCR. Both types of error can be attributed to human error. The error rates observed were similar to the technical error previously reported for molecular serotyping of *Salmonella* (12). Cross-contamination errors were identified by the detection of a common antigen or serovar in many adjacent samples, and technical errors were identified by a partial antigenic formula (i.e., particularly when missing the phase 1 flagellar antigen) or a formula with no match in the WKL scheme. Check&Trace provided the message "DNA recognition not OK, please reprocess sample from the sampling step." Traditional serotyping was considered the gold standard for the purpose of this study, although there is a known error rate and variability between laboratories associated mainly with the inability to detect the phase 2 flagellar antigen and differences in antisera quality (18). The panel of isolates used in this study was a well-characterized validation panel, and repeat serotyping was completed when there were consistent discrepancies across the four molecular methods.

Other evaluations of these methods include that of Beaubrun et al. (13), who compared Check&Trace and xMAP to a PCR-based method. The 365 cilantro samples containing *S. enterica* serovars Montevideo, Newport, Saintpaul, and Tennessee were successfully identified by Check&Trace, but results for serovars Newport and Saintpaul were inconclusive by xMAP. Another study tested 233 isolates that represented more than 52 serovars to compare Check&Trace and xMAP to traditional serotyping (19). They found that Check&Trace correctly identified 150 isolates (64%), and the xMAP assay correctly identified 181 isolates (78%). In another study, Wattiau et al. (6) tested 754 strains using Check&Trace and found that it performed almost as well as traditional serotyping, although it had difficulty identifying uncommon serovars.

Each method has its own advantages and limitations. The SGSA had high throughput ($n = 96$) and correctly identified the highest number of isolates and serovars in this study. However, it is not currently commercially available, but its components have been published, it is ISO 17025 accredited, and this technology can be readily adapted into a laboratory setting. While it currently detects the smallest number of serovars, it has the theoretical capability of identifying more than 2,000 serovars, although not all of these have been validated. In addition, it ambiguously identifies serovar Enteritidis and other groups of serovars that have extremely similar antigenic formulae (12). Work is under way to develop a new SGSA layout to provide this additional resolution as well as additional serotyping capability.

Salm SeroGen is also high throughput ($n = 96$), and, in addition to serovar identification, it evaluates the presence of antimicrobial resistance genes, which was not evaluated in this study. Although Salm SeroGen claims to identify the largest number of serovars, it is the second most costly kit and correctly identified the lowest number of isolates in this evaluation. In addition, Salm SeroGen does not perform well on less common serovars and reports a serovar regardless of the pass/fail of controls, which is not intuitive and requires expertise in *Salmonella* serotyping and antigenic formulae. Similarly to the SGSA, Salm SeroGen does not unambiguously identify the serovars Enteritidis, Dublin, Senftenberg, Panama, and Hadar from highly homologous serovars.

We found the Check&Trace software easy to use, but contrary to the other methods, it is low throughput. It processes samples in a tube format, which can handle up to three samples per tube. In addition, Check&Trace does not identify an antigenic formula or disclose how it identifies serovars. It is not based on somatic and flagellar gene identification (which would provide additional flexibility in identification of partial antigenic formulae or new serovars), as are the other three methods. Last, Check&Trace is the most costly kit.

xMAP is high throughput ($n = 96$), but significant manual analysis and interpretation are required. The raw data are interpreted manually to identify positive probes. Probes with median florescent intensity (MFI) values greater than 1,000 and/or a signal/noise ratio of six or greater are considered positive. Positive probes were cross-referenced to the White-Kauffmann-Le Minor scheme (5), which requires expertise in serotyping. In addition, xMAP had the highest number of technical repeats. In our hands, this kit was the hardest to use with respect to calibration of equipment and interpretation of results.

Two samples, serovars Borreze (54:f,g,s:-) and Crossness (67:r:1,2), were misidentified by all four methods. Borreze was misidentified as serovar Agona using Check&Trace (10), and Crossness was misidentified as Heidelberg by xMAP (20). Serogroups O:54 and O:67 are not common and not targeted by the four methods. Serogroup O:67 is a minor variant of serogroup B, which resulted in the identification of serovar Heidelberg (B:r:

TABLE 5 *Salmonella* serovar coverage, processing time from pure culture and cost of four molecular serotyping methods compared to traditional serotyping and whole genome sequencing

Method	No. of serovars the kit claims to detect	Processing time (days) (batch of 96 samples)	Cost per sample (CAD) (without labor)
SGSA	57 (12)	1–1.5	$30.47
Salm SeroGen	132 (User Guide version 1 issued February 2013)	1–1.5	$41.13
Check&Trace	102 (User Manual issued December 2011)	1–2[a]	$42.60
xMAP	75 (plus 25 partial) (kit insert issued June 2012)	1–2	$24.89
Serotyping	NA	2–3[b]	$15.30 to $42.79
WGS	NA	3–5	$131.87

[a] Processing time based on a batch of 36 samples.
[b] Processing time based on a batch of 50 samples.
NA, not applicable.

1,2). The O factor 54 is plasmid determined, and serogroup O:54 strains also have a chromosomally encoded *rfb* cluster that encodes other serogroups, which can result in the coexpression of multiple serogroups (20). The isolate of serovar Borreze tested in this study must have had the gene encoding serogroup B, resulting in the identification of serovar Agona (B:f,g,s:[1,2]).

At the time the molecular kits were evaluated in this study, they cost between $25 and $43 Canadian dollars (CAD) per sample (Table 5), and they generally took 1 to 2 days to complete from pure culture for a batch of 96 samples, except Check&Trace, which was based on a batch of 36 samples. The cost of traditional serotyping *Salmonella* including culturing in Canadian reference laboratories varies from $15 to $20 CAD per sample (S. Christianson and G. Arya, personal communication, May 2016). The European Centre for Disease Prevention and Control (ECDC) recently reported a median cost of €29 ($43 CAD) to serotype a common *Salmonella* serovar, including culturing and higher costs associated with more difficult cases (21). There are reports of traditional serotyping costs as high as $185 USD (22), but it is difficult to assess this wide range in costs as it depends on methods used, whether antisera is purchased or produced in-house, and whether the estimate includes labor. The cost of WGS is up to three times more expensive than molecular serotyping methods at $132 CAD (A. Reimer, personal communication, April 2016). These Canadian WGS costs are similar to the €91 ($134.28 CAD) reported in Europe (21). The processing time from pure culture using molecular methods is reduced from 2 to 3 days from pure culture for traditional serotyping to 1 to 2 days, and the throughput is significantly increased for common serovars. The processing time for in-house WGS is 3 to 5 days.

For nearly 100 years, identification of *Salmonella* has been done by serotyping, which identifies the somatic and flagellar antigens for the identification of a serotype. Serotyping requires highly trained technologists and expensive antisera. Globally, there is a move toward implementation of molecular diagnostics as an alternative for *Salmonella* serotyping for improved detection throughput. Although the future of *Salmonella* typing will rely on WGS, it will not be adopted in routine diagnostic laboratories for public heath purposes until challenges, including cost, data analysis, and interlaboratory comparability, are resolved. Until such time, the use of molecular serotyping will provide a valuable high-throughput alternative to traditional serotyping for the identification of prevalent *Salmonella* serovars. Each method evaluated in this study has its own advantages and disadvantages. They continue to classify into *Salmonella* serovars, which is important for continuity with historical serotyping data. The ideal molecular alternative method for the identification of *Salmonella* serotype should be rapid, nonsubjective, and high throughput. This study was the first to thoroughly evaluate all commercially available methods for molecular serotyping of *Salmonella*.

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