Acute Gastrointestinal Illness (AGI) Study: LABORATORY SURVEY

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by

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SUMMARY

This survey of community and hospital laboratories is one of the elements of a study of acute gastrointestinal illness (AGI) in New Zealand. The other elements are a survey to determine the prevalance of AGI in the community, and an investigation of the incidence of AGI-related visits to General Practitioners.

The overall objectives for the AGI study are:

- To determine the magnitude and distribution of self reported AGI in the New Zealand population;
- To estimate the burden of disease associated with AGI;
- To describe and estimate the magnitude of under-ascertainment of AGI at each stage in the national communicable disease surveillance process; and,
- To identify modifiable factors affecting under-ascertainment that, if altered, could reduce case loss throughout the AGI component of the surveillance system.

The laboratory study is described in the project specification as:

- A survey of all community microbiological laboratories to describe and quantify the under-ascertainment of AGI at the phase when a stool specimen is submitted for analysis for enteric pathogens. Variables to be studied will embrace laboratory protocols including criteria for examining for specific pathogens and methods used, and reporting practices to both practitioners and Medical Officers of Health.

Through examination of several sources, 45 community and hospital laboratories that performed relevant analyses of faecal samples were identified. All except one agreed to participate in the survey. In addition, the two ESR Public Health Laboratories were asked to participate. This gave a total of 46 laboratories to which the survey was sent.

The survey instrument was based on that used for a survey of the same laboratories for Campylobacter testing in 2005 (which in turn was based on a survey conducted by the Centers For Disease Control in the United States). This template was expanded to cover the broader range of pathogens to be covered by the AGI study, as well as additional information such as testing criteria. The methodology information requested was reduced to a brief description. Minor revisions were made after a pilot survey of five hospital and community laboratories.

The revised survey was sent on 29 June 2006 to the remaining 39 hospital and community laboratories, as well as the two ESR laboratories. When the survey was declared closed (29 September 2006), replies had been received from 28/39 community and hospital laboratories, and both ESR laboratories. Combining the pilot and full surveys, this meant that there were 46 relevant laboratories, from which 35 replies were received.

The response rate for this survey was lower than expected, given that each laboratory had been contacted directly and agreed to participate. From anecdotal comments by non-responding laboratories, it appeared that they may have been inhibited from responding by the extremely competitive commercial environment which pervaded the laboratory sector during the survey period.

However, the different laboratory types are well represented, and good geographical coverage suggest that the results are representative of the sector as a whole.

As might be expected, approximately 77% of the samples derive from people presenting to primary health care providers, while the majority of the remainder are from patients in hospitals.

The majority of samples are accompanied by a test request form, but the testing requested is often in general terms (e.g. "culture"). Laboratories are not informed of the individuals symptoms for all samples, but if they are supplied, the information is more likely to influence the testing performed. Responses to testing criteria for individual types of pathogens suggests that the patient's age, travel history, and condition of the specimen were more influential than the symptoms themselves.

Discarding of specimens appears to be infrequent, and occurs mostly because the sample is unsuitable for testing, either physically or administratively.

It appears that, unless instructed otherwise, laboratories will test faecal specimens for the bacterial pathogens Campylobacter, Salmonella, Yersinia and Shigella, and the parasites Giardia and Cryptosporidium. Tests for *E. coli* O157, Vibrio, rotavirus and *C. difficile* toxin might also be performed depending on the clinical symptoms or age of the patient, and Aeromonas can be detected as part of routine culture for the other bacterial pathogens. The pathogenicity of Aeromonas remains to be determined.

Testing for the bacterial pathogens Campylobacter, Salmonella, Yersinia and Shigella is conducted at least five times more frequently than other bacteria. Samples are ten times less likely to be tested for STEC including *E. coli* O157, and *Vibrio* spp., while testing for *Listeria* spp., *Bacillus* spp., *Clostridia* spp., and *Staphylococcus aureus* is performed on very few samples. When the survey of GPs is completed, a comparison between their expectations of testing and actual tests can be conducted.

In terms of criteria for bacterial testing, the majority of testing is conducted as routine screening procedures, with some tests performed when requested. A number of laboratories reported that some tests were not available; principally *Enterobacter sakazakii*, *Bacillus* spp., *Clostridia* spp. and *Staphylococcus aureus*. Laboratory decision making was most frequently reported for STEC (decision based on bloody diarrhoea, clinical symptoms, or age of patient) and *Vibrio* spp. (decision based on liquid/fluid sample, clinical details, travel or seafood consumption).

The methods reported for detection of bacteria indicate that there is considerable variation across laboratories in terms of enrichment, agar, and incubation temperatures. None of the methods reported would be ineffective, but the experience of the laboratory worker would be a factor in the recognition of some bacteria.

Only a small proportion of faecal samples are tested for enteric viruses. Approximately 10% of samples are tested for rotavirus, about 1.5% for adenovirus, and less than 0.5% for norovirus. Decision making for testing for enteric viruses was evenly split between request or laboratory criteria. As expected, the laboratory criteria for rotavirus testing was largely based on the age of the patient. Testing of samples from young children was most common, although the maximum age of the children varied considerably.

Testing of faecal samples for norovirus most commonly occurs in an outbreak situation, and only a few samples are likely to be tested as indicative. This will therefore miss the endemic burden of norovirus infection.

Methods for detection of enteric viruses appeared to be suitable.

Responses to questions regarding parasite and protozoa testing indicated that approximately a quarter of all faecal samples were tested for ova, while approximately a third were tested for Giardia and Cryptosporidium. Tests for a wide range of other parasites are conducted, but only on a small number of samples (perhaps 1% of the total).

Testing for parasites and protozoa was reported as routine in only a few laboratories; most perform this type of testing only on request, while some laboratories report making their own decisions. Based on reported sample numbers however, parasite testing is requested frequently. The laboratory criteria used were mostly related to clinical details, while recent overseas travel was important for conducting ova testing, and the sample being from a child was important for Giardia and Cryptosporidium testing.

There was considerable variation in the testing methods reported for parasites and protozoa. Laboratory worker experience would be a key factor in successful detection of the pathogens, particularly for microscopy methods.

Testing of faecal samples for toxins is rare. Testing is most often performed for *C. difficile* toxin, with perhaps 7% of samples tested. This test is more likely to be performed on samples from hospitalised patients, because *C. difficile* is a common cause of colitis and/or diarrhoea following antibiotic intake in a hospital or care facility.

A high proportion of laboratories (31/35) store samples, with the most common time period being a week. Refrigerated storage was most common.

It is expected that for a large number of samples, no pathogen will be detected. Apparently laboratory systems are not well set up to provide this type of information, but data from 17 of the laboratories indicate that no pathogens are detected in two thirds or more of samples.

Most of the laboratories who responded indicated that no further testing was performed on samples from which no pathogen was identified, and no samples are referred to the ESR Public Health Laboratories. From the remaining laboratories, the number of samples subjected to further testing was very low, and most likely to be from children.

The referral of isolates to the ESR Enteric Reference Laboratory was routine for nearly all laboratories in the case of isolation of Salmonella, Shigella and STEC, and for approximately half the laboratories when *Vibrio* spp. or Yersinia isolates were obtained.

Healthlink (a computer network) or print reporting formats were most common for sending results to GPs and Public Health Units. Direct contact (by telephone) between the laboratory and test requestor would most often occur to clarify testing requirements, or report results of public health significance (e.g. the isolation of pathogens with serious adverse outcomes).

To construct an estimated national overview for New Zealand, it is necessary to extrapolate from the information obtained by the survey. The calculations are based on the following:

Faecal samples submitted:

> A total of 184,252 samples were reported by 34 laboratories. Based on publicly available information, the remaining laboratories were assigned to a category. Missing were results from:

6 Hospital laboratories
5 Community laboratories
1 Hospital and community laboratory

Using the reported survey results, this suggests total samples for New Zealand of 218,970 (using median values) or 256,471 (using mean values). The differences between the mean and median values indicate that the distributions for sample numbers are skewed. The largest contributor to sample numbers are the community laboratories. Since there were several major community laboratories that did not respond to the survey, the value derived from the means is considered more reliable.

A total of 256,471 samples represents a rate of 0.06 samples per person per year for a New Zealand population of 4,098,900 (based on Statistics New Zealand population estimates for 2005).

Faecal samples discarded:

Few laboratories were able to provide quantitative data for this question. Of the laboratories that did respond, the answers were 0% (7 laboratories), <1% (4 laboratories), 5.5%, 7.1%, <13.6%. The mean value from these responses is approximately 2%.

Faecal samples tested:

Based on the above information, this suggests that 251,341 samples were tested.

Pathogen found:

Based on review of data on community derived samples, it is estimated that pathogens were found in approximately 20% of samples. This represents up to 50,000 samples (0.01 samples per person per year).

New Zealand and overseas reports suggest that approximately 25% of patients (over 5 years) with AGI symptoms presenting to general practitioners (GPs) in New Zealand are requested to provide stool samples. Using the estimated 256,471 stool samples submitted in 2005, of which an estimated 77.1% derived from primary healthcare providers, there may be up to 791,000 GP consultations by people in New Zealand with AGI symptoms. This estimate should be treated with caution however; this survey did not ask laboratories to differentiate between stool samples submitted by patients with AGI and those submitted for other reasons.

There is considerable variation in the methods used for all the pathogens examined in this study. It is likely that if pathogens are present, then they will be present in high numbers and sensitivity (and enrichment, for bacterial pathogens) should be less important. Therefore variation in methods may not be a significant factor in detection.

1 INTRODUCTION

This survey of community and hospital laboratories is one of the elements of a study of acute gastrointestinal illness (AGI) in New Zealand. The other elements are a survey to determine the prevalance of AGI in the community, and an investigation of the incidence of AGI-related visits to General Practitioners. The study is being conducted by the Institute for Environmental Science and Research (ESR) for the New Zealand Food Safety Authority (NZFSA).

The overall objectives for the AGI study are:

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1.1 Previous Survey on Testing for *Campylobacter* spp.

In 2005, a survey was conducted by ESR of community and hospital laboratories concerning stool specimens and methods used for the isolation and diagnosis of infection with *Campylobacter* spp. The survey was prompted by ESR's participation in the International Collaboration on Enteric Disease Burden of Illness Studies. This survey provided the AGI laboratory study with valuable information on relevant laboratories, as well as an initial questionnaire template. During the analysis phase of the AGI laboratory survey, the opportunity was taken to compare relevant results with those obtained from the Campylobacter survey.

2 METHODOLOGY

2.1 Participating Laboratories

A list of the 78 laboratories to which the 2005 Campylobacter survey was sent was obtained from Dr. Chris Pope. This was compared to a list of registered medical laboratories on the website of International Accreditation New Zealand (IANZ) to check that no laboratories had been missed (https://secure.ianz.govt.nz/scripts/IANZWebSearch/IANZWebSearch.exe/LabMed). The list was also compared to a list of diagnostic microbiology laboratories held by the Communicable Disease Group at ESR for linking laboratories with surveillance data. No additional laboratories were identified through these checks.

As the diagnostic microbiology laboratory sector had been in considerable flux prior to the survey period, each of the 78 laboratories was contacted by telephone to determine:

- Current status of the laboratory in terms of continued operation;
- Whether the laboratory performed analyses or acted as a sample collection point;
- Willingness to participate in the survey; and,
- Contact person and confirmed contact details.

As a result of this process, 45 community and hospital laboratories that performed relevant analyses were identified. All except one agreed to participate in the survey. In addition, the two ESR Public Health Laboratories were asked to participate. This gave a total of 46 laboratories to which the survey was sent. A third ESR laboratory, the Norovirus Reference Laboratory (NRL), receives specimens referred from other laboratories throughout New Zealand. This laboratory supplied additional data on norovirus testing, and these data have been incorporated into the sections of this report specific to norovirus.

2.2 Survey Instrument

The template for the questionnaire design was the 2005 Campylobacter survey (which in turn was based on a survey conducted by the Centers For Disease Control in the United States). This template was expanded to cover the broader range of pathogens to be covered by the AGI study, as well as the additional information such as testing criteria. The methodology information requested was reduced to a brief description. The template was peer reviewed by several staff in the Communicable Diseases group at ESR, and in the two ESR Public Health Laboratories, as well as benefiting from the diagnostic laboratory experience of one of the authors of this study (Philippa Bridgewater, Aotea Pathology).

2.3 Pilot Survey

The survey was sent to five laboratories as part of a pilot exercise on 5 May 2006 (participation by these laboratories had been confirmed by telephone). All five laboratories replied, although one reply was incomplete. As a result of this exercise, some questions were slightly amended to clarify the information sought.

The finalised survey instrument is attached in Appendix 1.

2.4 Full Survey

The revised survey was sent on 29 June 2006 to the remaining 39 hospital and community laboratories, as well as the two ESR laboratories, with a request for response by 19 July. Following this date, in an effort to increase the number of responses, on 17 August 2006 a non-monetary reward was sent to the respondent laboratories (excluding the ESR laboratories), and this was also offered in a letter to the non-responding laboratories. One additional survey reply was received following this exercise.

When the survey was declared closed (29 September 2006), replies had been received from 28/39 community and hospital laboratories, and both ESR laboratories. Combining the pilot and full surveys, this meant that there were 46 relevant laboratories, from which 35 replies were received. Additional data on norovirus testing was obtained from the ESR NRL, but these data have been excluded from the general analysis and are presented separately where appropriate.

3 RESULTS

3.1 Analysis of Laboratory Responses

The number of laboratories that responded to the pilot and full surveys are indicated in Table 1.

Table 1: Laboratory response rate for pilot and full surveys

Survey	No. surveyed	No. respondents	Response rate
Pilot survey	5	5	100.0%
Full survey	41	30	73.2%
Total surveyed	46	35	76.1%

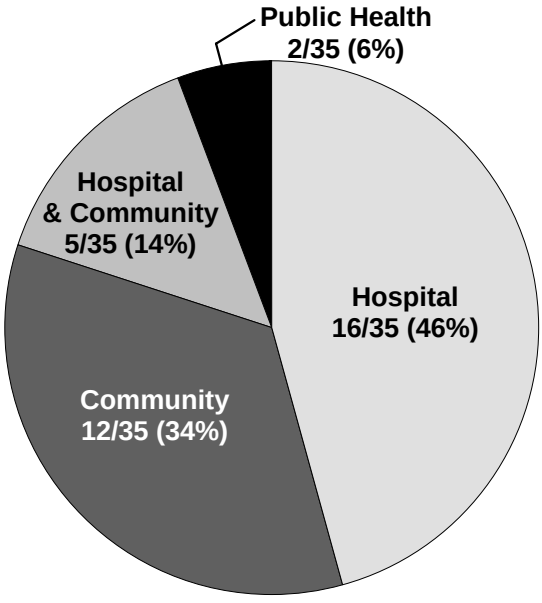
3.2 General Laboratory Information

3.2.1 Laboratory description

Q1: Which of the following best describes your laboratory? (Hospital-based, Community diagnostic, Public health)
Responses: 35/35 (100%)

The self-selected descriptions are summarised in Figure 1.

Figure 1: Self-selected classification of laboratory type for all respondents



3.2.2 Regional coverage

Q2: Approximately, what geographical region(s) is served by your laboratory?
Responses: 35/35 (100%)

Geographical coverage is presented as numbers of respondent laboratories for three regions:

> Northern (Northland, Waitemata, Auckland, Counties Manukau, Waikato, Bay of Plenty, Lakes, Tairawhiti District Health Boards): 17/20 laboratories responded
>
> Central (Taranaki, Hawkes Bay, MidCentral, Whanganui, Hutt, Capital and Coast, Wairarapa District Health Boards): 8/13 laboratories responded
>
> South Island (Nelson Marlborough, West Coast, Canterbury, South Canterbury, Otago, Southland District Health Boards): 8/11 laboratories responded

The two Public Health Laboratories have been excluded from this analysis.

3.3 Stool Specimens

3.3.1 Stool specimen collection

Q3: How many stool samples did your laboratory receive during the period 1 January to 31 December 2005?
Responses: 34/35 (97.1%)

Q4: How many individuals did these samples come from?
Responses: 16/35 (45.7%). Many indicated they were unable to produce these data.

The number of stools collected by the responding laboratories, and the number of individuals from which stools were collected, are summarised in Table 2.

Note that almost all laboratories provided data for total stools, but only 16 laboratories provided data for individuals. Only where a laboratory has provided data for both Q3 and Q4 is it possible to calculate a stools/individual value (Table 3). The ratios in Table 3 for community, and hospital & community laboratories are derived from data from 3 laboratories each, whereas the ratio for hospital laboratories is from data reported by 8 laboratories. The ratio for community laboratories is higher than for the other two types of laboratory. This may be due to GPs requesting multiple samples; but as only a small number of laboratories provided numbers, this suggests that these data should be treated with caution.

Table 2: Number of stools, and number of individuals from which stools were collected, by laboratory type in 2005

Lab type:	Hospital only	Community only	Hospital & Community	Public Health	All Laboratories
Stools					
Responses[a]	15/16	12/12	5/5	2/2	34/35 (97%)
Total (%[b])	32,255 (17.5%)	134,379 (72.9%)	16,644 (9.0%)	974 (0.5%)	184,252
Mean	2,150	11,198	3,329	487	5,419
Median	858	5,717	985	487	2,076
Max	11,500	56,997	13,162	867	56,997
Min	136	247	409	107	107
Individuals					
Responses[a]	8/16	3/12	3/5	2/2	16/35 (46%)
Total (%[b])	6,288 (10.3%)	52,632 (86.1%)	1,579 (2.6%)	601 (1.0%)	61,100
Mean	786	17,544	526	301	3,819
Median	578	10,356	550	301	579
Max	2,854	34,276	620	505	34,276
Min	76	8,000	409	96	76

[a] Note response rate for provision of data for stools and individuals. These data cannot be directly compared.

[b] Percentages based on total stools or total individuals from all laboratories that supplied data.

Table 3: Stool specimens per individual for each laboratory type

Lab type:	Hospital only	Community only	Hospital & Community	Public Health	All responses
Responses*	8	3	3	2	16
Mean	1.3	1.9	1.3	1.4	1.4
Median	1.3	1.7	1.3	1.4	1.4
Max	1.9	2.6	1.6	1.7	2.6
Min	1.1	1.5	1.0	1.1	1.0

* Number of laboratories that provided data for Q3 and Q4, from which values are calculated

An additional 356 stools were tested by the NRL for norovirus. A significant portion of these specimens were referred from other laboratories, and may be included in the data presented above. Some of these specimens were already confirmed as being norovirus-positive, and were referred to the NRL for sequencing only.

3.3.2 Rate of faecal sample provision in the community

The rate of faecal sample provision was calculated by carefully reviewing the sample numbers reported, the laboratory type and regional District Health Board (DHB) populations. It is recognised that laboratories will not exactly serve a specific DHB region, and patient mobility will also affect sample provision, but until figures are available for the populations served by each laboratory the DHB population figures (based on Statistics New Zealand population estimates for 2005) have been used as an interim value.

The intention was to calculate the rate of sample provision by people in the community, including those from primary and occupational healthcare providers and public health units, while excluding hospital patients who are likely to have a higher rate of sample provision, and/or different transmission routes and pathogens present. The percentages of sample sources (Question 5) were used to estimate the non-hospital number of samples for each laboratory.

For each region, the presence of respondent community laboratories was reviewed. In DHBs where no community laboratory responded, the rate was not calculated. For one major laboratory, which serves three DHB regions, the population numbers were amalgamated. For the lower North Island and Wellington, three DHBs and laboratories were also amalgamated, as overlap was considered likely.

Based on the available data and responding laboratories, rates were calculated for populations covered by 16 of the 21 DHBs. The rate of community faecal sample provision in those regions ranged from 0.02 – 0.06 samples per person per year for 15 regions, with one region (Waikato) having a higher rate of 0.1 samples per person per year. The reason for the apparently higher rate in the Waikato is unknown.

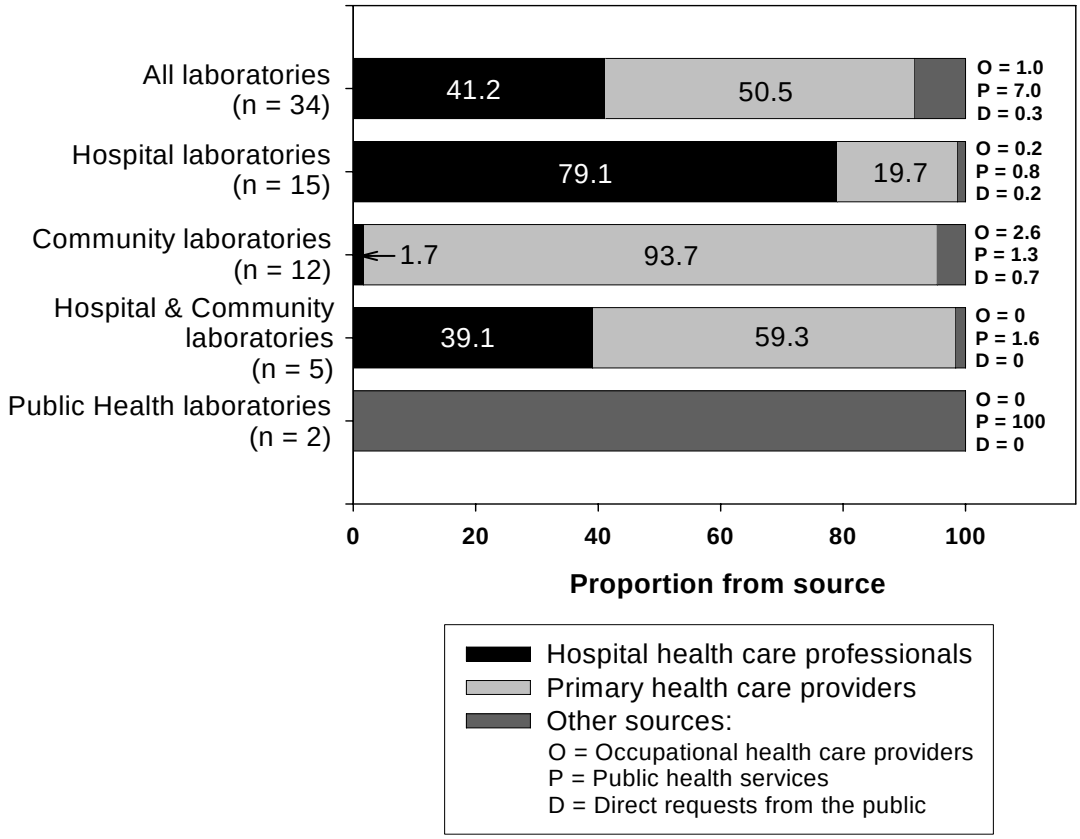
3.3.3 Stool specimen sources

Q5: Approximately, what proportion of your stool specimen testing is requested by the following sources? (Hospital health care professionals, Primary health care providers, Occupational health care providers, Public Health Services, Direct requests from the public)
Responses: 34/35 (97.1%).

The proportion of stool specimens received from each source, by laboratory type, is presented in Figure 2.

Figure 2: Source of stool specimens received by laboratories according to laboratory type

(n = number of laboratories responding to Q5)



The data summarised in Figure 2 has been combined with the numbers of samples reported in Table 2, to provide the numbers and percentages derived from each source shown in Table 4. The majority of samples are derived from people presenting to primary health care providers.

Table 4: Numbers and percentage of samples derived from each source

Laboratory type	Total samples	Samples from hospital professionals	Samples from primary health care providers	Samples from other sources
Hospital	32,255	25,514	6,354	387
Community	134,379	2,284	125,913	6,181
Hospital and community	16,644	6,508	9,870	266
Public Health Laboratories	974	0	0	974
Total (%)		34,306 (18.6)	142,137 (77.1)	7,809 (4.2)

3.4 Decision Making

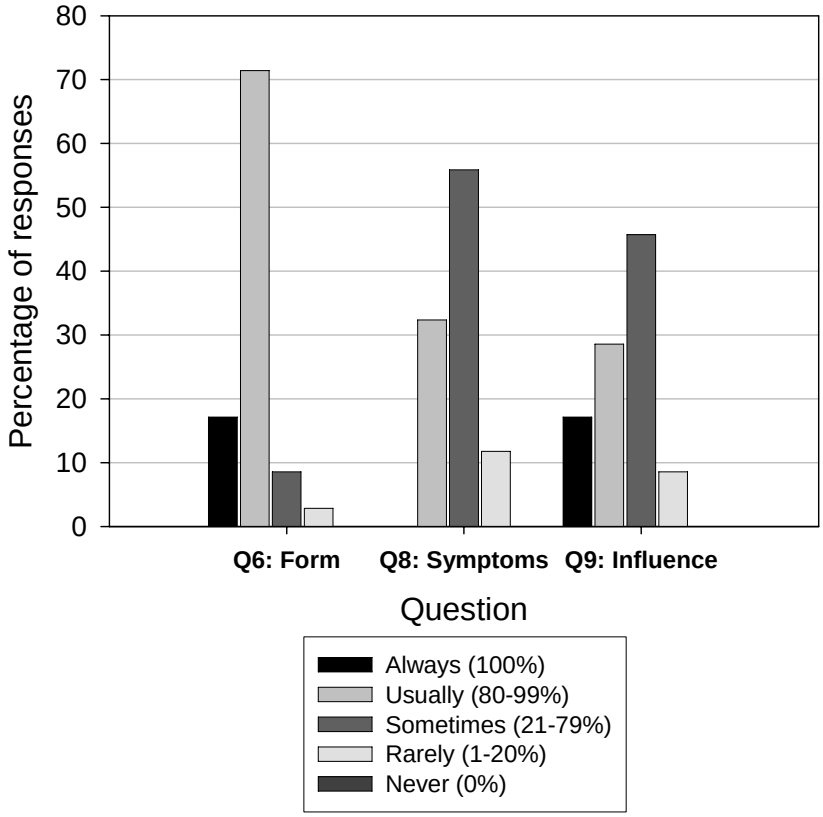
3.4.1 Specimen forms and laboratory response

Q6: Do samples arrive with the required testing specified on a standardised laboratory form?
Responses: 35/35 (100%)

Q8: Is the laboratory made aware of a patient's symptoms?
Responses: 34/35 (97.1%)
Q9: Does knowledge of a patient's clinical details influence your testing regimen?
Responses: 35/35 (100%)

The responses to these questions are summarised in Figure 3.

Figure 3: Decision-making for stool testing: The regularity of tests being specified on a form ('form'), whether the laboratory is made aware of patient's symptoms ('symptoms') and whether clinical details influence the testing regime ('influence')



3.4.2 Standard tests

Q10: If you receive a sample where the only request is for "faecal (or enteric) pathogens", what tests would your laboratory perform on the sample?
Responses: 35/35 (100%). Of these, one responded with 'not applicable', and the two Public Health laboratories have been excluded as they do not perform routine screens but make analytical decisions based on information received.

The remaining 32 responses for this question have been separated into those that were 'non-specific', i.e. no pathogens specifically listed, and those where pathogens were specified. For the latter group, the information has been further separated into bacterial pathogens, parasites, viruses and toxins.

Non-specific responses

A number of responses to this question did not specify, or only partially specified, the pathogens that would be tested for, particularly bacterial pathogens. These responses are summarised as follows:

- No specific pathogens identified – 5 responses: 'culture only', 'culture for enteric pathogens', 'microscopy (direct) and culture (routine)' or 'look for all routine enteric pathogens'.
- 'culture' or 'routine culture', with some detail on parasites, viruses or toxins (commonly rotavirus, *Giardia*, *Cryptosporidium* and *Clostridium difficile* toxin; see below) – 11 responses.
- 'culture' with some examples of bacteria given, but full range not listed – 2 responses, specifically:
    - 'Generally culture would be carried out for faecal pathogens including *Campylobacter'*
    - 'Full culture including *E. coli* O157 and *Yersinia'*

Bacterial pathogens

Thirteen responses specifically listed the bacterial pathogens that would be tested. These responses are summarised in Table 5.

Table 5: Bacterial pathogens that faecal specimens are analysed for, as specified by thirteen laboratories

Pathogen	Respondent													
	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
Salmonella	●	●	●	●	●	●	●	●	●	●	●	●	●	13
Shigella	●	●	●	●	●	●	●	●	●	●	●	●	●	13
Campylobacter	●	●	●	●	●	●	●	●	●	●	●	●	●	13
Yersinia	●	●	●	●	●	●	●	●	●	●	●	●	●	13
E. coli O157	●	●	●		●			●				●	●	7
Aeromonas	●	●	●	●								●	●	6
Vibrio	●												●	2
Plesiomonas		●	●											2

Salmonella, Shigella, Campylobacter and Yersinia appear to be the most common bacterial pathogens tested for as part of routine faecal examinations, as also indicated by responses such as 'if no clinical details - culture for Salmonella/Yersinia/Shigella/Campylobacter and Giardia/Crypto testing' and 'routine culture for Salmonella, Shigella, Campylobacter, Yersinia'. The testing for other bacterial pathogens was often associated with certain criteria. For example, four responses specified that the criteria for *E. coli* O157 testing was a specimen that was bloody or from a child, and one response mentioned that they would also do an 'EIA test for EHEC'. One response indicated that Vibrio testing would be undertaken if the 'clinicals were suggestive'. There is some suggestion that Aeromonas and Plesiomonas are not specifically tested but might be isolated through the routine testing methods used for Salmonella, Shigella, Campylobacter and Yersinia or while isolating for other bacterial pathogens. This is supported by information from the methods section of the survey (Section 3.5) and one response to Q10 that stated 'Aeromonas and

Plesiomonas can be isolated from CTSMAC plate' (CTSMAC is a routine solid agar used for isolation of *E. coli* O157:H7).

Parasites

Thirteen laboratories indicated that they would perform some sort of testing for parasites ('parasitology'). Of these only one did not specify any particular parasites and the remaining 12 specified Cryptosporidium and Giardia. No other parasites were listed in any response. An additional laboratory specifically stated that parasites would not be tested. Within the responses, there was an association between non-specific answers with regard to bacterial pathogens (i.e. 'culture' or 'routine culture', see above) and the specification of *Cryptosporidium* and *Giardia* (8 responses), suggesting that these two parasites are often part of routine faecal examination.

Viral pathogens

Thirteen laboratories indicated that they would perform some sort of testing for viruses. Of these, 12 specified rotavirus only, and one rotavirus and adenovirus. All but one indicated that testing for rotavirus or adenovirus depended on the patient's age (e.g. from a child or patient less than five years old). Other testing criteria listed by four of these laboratories were if the specimen was 'loose or fluid' or testing was considered based on the 'clinical details'.

Toxins

Three responses mentioned testing for toxins, all for *Clostridium difficile* toxin. The responses indicated that *C. difficile* toxin testing was considered if the specimen was from patients hospitalised more than three days (two responses), or by clinical details or age (one response).

Some examples of responses

- Generally culture would be carried out for faecal pathogens including Campylobacter, if the patient has been an in-patient for >3 days then *Cl. difficile* testing will be offered instead.
- Too many variables to answer well! Would depend on patient's age and requesting source. E.g. <5 yrs get rotavirus, Giardia/crypto automatically. Immigration would get other parasites. Also depend on specimen, i.e. PVA.
- Routine bacterial culture and rotavirus depending on age (ie no parasite work and no toxin testing).
- If no clinical details - culture for Salmonella/Yersinia/Shigella/Campylobacter and Giardia/Crypto testing.
- Culture for Salmonella, Shigella, Campylobacter, Yersinia, Aeromonas, Vibrio, All children also have rotavirus test, and *E. coli* O157 plate.
- Culture for Salmonella, Shigella, Campylobacter, Vibrio, Yersinia, Aeromonas, O157 *E. coli*. EIA test for EHEC, if under 5 years old also test for rotavirus and adenovirus.
- Salmonellae, Shigellae, Campylobacter, Yersinia, *E. coli* O157 (Aeromonas and Plesiomonas can be isolated from CTSMAC plate) i.e. routine examination culture and wet prep for WBCs.
- Culture for Salmonella, Shigella, Campylobacter, Yersinia, Aeromonas, Plesiomonas, Giardia and Cryptosporidium antigen. *E. coli* O157 if bloody specimen or child <12. TCBS for Vibrio if clinicals suggestive. rotavirus if loose spec on child <5 yrs.

Summary of responses to Q10

From the consolidated information, where no directive is given, it appears to be common practice for laboratories to test faecal specimens for the bacterial pathogens Campylobacter, Salmonella, Yersinia and Shigella, and the parasites Giardia and Cryptosporidium. *E. coli* O157, Vibrio,

rotavirus and *C. difficile* toxin might also be tested depending on the clinical symptoms or age of the patient, and Aeromonas can be detected as part of routine culture for the other bacterial pathogens.

When hospital laboratories were compared with laboratories categorised as community (with or without hospital/community laboratories included) there were only minor differences in the reported pathogen testing conducted as a result of routine procedures or "on request" testing. The only differences occurred in the routine or request testing of the more uncommon bacteria (Aeromonas, Vibrio, Plesiomonas, Enterobacter, Staphylococcus) which were usually more frequently reported by hospital laboratories. Conversely testing for *Clostridium* spp. was reported only by community or hospital/community laboratories (4/17) and only "on request".

Testing for "other enteric viruses" was reported only by hospital laboratories (5/16) and only "on request".

Note that Public Health Laboratories were excluded from these comparisons.

Testing for *C. difficile* toxin was most commonly reported as being tested "on request" by hospital laboratories, but several community laboratories also reported conducting this test.

3.4.3 Examples of specimen forms

Question 7 requested examples of specimen forms if laboratories were able to supply them. Twenty-three laboratories supplied over 200 anonymous specimen forms. The majority were standard laboratory forms, though a number were letters from the referring doctor. Most standard forms provided a tick-box for indicating that the sample was a faecal specimen, though in many cases it was not used and this information was recorded elsewhere on the form.

The information important for decision-making regarding testing of the specimen are the tests required and relevant clinical information.

Where letters were provided in place of a standard laboratory form, there was usually some specification of the tests required, though not always. In most cases, the tests specified were very general, e.g. 'faeces – culture', 'faeces pathogens' or 'microscopy and culture'. On very few occasions specific pathogens were listed, usually *C. difficile* toxin or Giardia in the examples provided. Clinical details were rarely included in the letters, but some listed specific information on the clinical symptoms and history of the patient, such as previous results from tests, symptoms like diarrhoea, vomiting or abdominal pain and if a suspect food had been consumed.

Most standard laboratory forms provided an area where the required tests could be specified. Some did not, and the test had to be written in any space available (possibly the wrong form had been used in these instances). A large number of forms limited the choices available to a very few generalised options, examples follow:

The referring person has the option of selecting from the following:

- 'Microscopy' and/or 'bacterial culture'
- 'Culture' and/or 'C diff'
- 'Cult', 'Rotavirus', 'C. difficile toxin', 'parasite' and/or 'occult blood'
- 'Culture', 'C. diff', 'Parasites' and/or 'Rotavirus'
- 'Culture/sens', 'Parasites/cysts/ova' and/or 'occult blood'
- 'Culture', 'Giardia/Crypto', 'Ova & parasites' and/or 'C. difficile'

- ‘Culture’, ‘Parasites’, ‘C. difficile’, ‘Rotavirus’ and/or ‘Virus’

Some forms only provided for selecting an option for ‘routine culture’, ‘diarrhoea screen’ or similar. In other instances there was provision to collect more specific information that might be used to guide the testing, such as a ‘history for parasite examination’, antibiotic use or recent/current drug therapy, overseas travel or a hospital admission date. Most forms provided an area where other tests could be specified, or more detail on the requested tests could be given, but this area was not often utilised in the examples provided. Where additional tests had been specified, the most common test was ‘MC & S’, which is an abbreviation used to request microbiology culture and antibiotic sensitivities of any isolates.

Most standard laboratory forms provided an area where supporting or relevant clinical information could be detailed, though these were rarely utilised in the examples provided. Where clinical details had been listed, some examples are ‘not on antibiotics’, ‘diarrhoea and vomiting’, ‘watery’, ‘high temperature’, ‘blood stained diaper’, ‘post-partum’ and ‘abdominal pain’.

One standard laboratory form attempted to link clinical circumstances with a testing regime, and was included in the examples provided by two hospital-based laboratories in the same region of New Zealand. This form has a section where, for a faecal specimen, the requesting person is able to select between ‘occult blood’, ‘*H. pylori*’ and/or ‘infectious diarrhoea’. If the latter is selected, then more extensive choices are presented in a matrix format linked to clinical symptoms and history. This matrix has been reproduced in Figure 4.

Figure 4: Reproduction of part of a standard laboratory form where the testing request is linked to clinical history and symptoms (Note: Yersinia is not included as "routine" on this form)

Select tests from options on the right based on clinical circumstances below:	Routine[1]	*Clostridium difficile*	*E. coli* O157:H7	*Vibrio* spp.	Giardia/Cryptosp.	Microscopic O&P[2]
Routine	☐					
Bloody stools or HUS	☐		☐			
Recent seafood	☐			☐		
Recent (<2 weeks) cytotoxics or antibiotics	☐	☐				
Diarrhoea starts after >72 hours in hospital		☐				
Recent camping, farm contact or tramping; child-care worker; or clinical syndrome suggests giardiasis	☐				☐	
Immunosuppressed[3] or diarrhoea >2 weeks	☐	☐			☐	☐
Recent travel to developing country	☐			☐[4]	☐	☐
Suspected outbreak[5]	☐					

HUS, haemolytic uraemic syndrome
[1] Routine includes *Campylobacter*, *Salmonella*, *Shigella*, *Plesiomonas*
and *Aeromonas* spp., rotavirus and *E. coli* O157:H7 if <5 yrs
[2] >95% of parasitic enteritis will be detected by the Giardia/Cryptosporidium antigen test – request microscopy for O&P
only if antigen test negative and symptoms continue
[3] If HIV/AIDS, consult ID or Micro Specialist
[4] If profuse watery diarrhoea (suspect cholera)
[5] Contact Public Health

3.4.4 Discarding of specimens

Q11: How many samples were discarded in 2005 before any testing was performed?
Responses: 29/35 (83%)

Q12: For what sorts of reasons are samples discarded without being tested?
Responses: 31/35 (89%) (one replied 'not applicable')

Of the 29 responses to Q11, eight laboratories replied that they were unable to provide data because it was not accessible or not known. Another eight laboratories suggest very small numbers of discarded specimens with the comments 'very few', 'not many', 'very occasional' and '<1%'. Seven laboratories replied with 'nil', 'none' or '0'.

For the remaining six laboratory replies the number discarded before testing (and a proportion calculated from the total specimens received at the laboratory), were as follows:

- <10 (<0.5%)
- <100 (<0.4%)
- Unknown – estimate 100 specs (0.8%)
- 159 (7.1%)
- <190 (<13.6%)
- About 300 (5.5%)

Reasons provided for discarding specimens (Q12) have been summarised in Table 6. The score reflects the number of responses that included the 'reason' in their reply.

Table 6: Reasons why laboratories will discard a faecal specimen without testing

Reason for discarding specimen	Score[a]
Problem with specimen:	
Too old[b]	12
Leaking	8
Inappropriate specimen e.g. Toilet paper, nappy, urine, received in fixative	5
Insufficient sample	4
Urine contamination	1
Formed (non-diarrhoeal) for culture	1
Problem with administration:	
Unlabelled or mislabeled	8
Mismatch between form & specimen	1
Lack of relevant clinical details for parasite examination	1
Received in inappropriate container	1
Multiple specimens:	
Multiple specimens from same allocation time[c]	12
Specimens received daily for >5 days	1
Additional responses:	
Hospital patients with >3 days length of stay[d]	4
When C. difficile requested but criteria not met e.g. formed stool, previous positive, old specimen, >3 specimens in one week	3
Test not done by laboratory e.g. norovirus	1

[a] The number of responses which listed this among the reasons for discarding a specimen.

[b] Three replies gave the specifics of their procedures: 'usually >24 hrs', 'more than 48 h old' and '>3 days'.

[c] The allocated time for 7 replies was specified as one day (i.e. only test one specimen per day, any additional specimens are discarded); two other replies included in this response were 'multiple stool samples in very short time interval' and '>2 samples sent for culture'.

[d] Reasons were as follows: 'Hospital patients with >3 days length of stay with no admitting symptoms of gastroenteritis', 'been in hospital for more than 3 days before diarrhoea started when only culture requested', 'patient been in hsp >72 hours - C. difficile only', 'parasites requested on patient who has been in hospital >3 days', 'samples requesting culture on patients who have been hospitalised for >3 days only have C. difficile toxin performed instead of culture'.

3.5 Bacterial Pathogens

Section F asked participants to provide information on the number of faecal specimens tested during 2005 for bacterial pathogens, and for each pathogen what criteria is used to decide if that pathogen should be tested for and the methods used to test the specimens.

3.5.1 Number tested

Q13.1-Q25.1: Participants were asked to provide the number of faecal samples tested in 2005 for 13 specific bacterial pathogens.

Q26.1: Participants were asked to provide the number of faecal samples tested in 2005 for any other bacterial pathogen not specified in Q13-Q25, and to specify that bacterial pathogen.

Table 7 summarises the number of faecal specimens tested for each bacterial pathogen where an absolute value of one or greater was recorded. To improve the data set, some responses were amended to produce absolute values, e.g. 'approx. 400' was amended to '400', '10-20' was amended to '15'. Only one laboratory indicated that they tested specimens for 'other enteric bacteria'; they tested for Vancomycin resistant *Enterococcus* spp., but did not provide the number of specimens tested during 2005.

The numbers of samples tested for each individual pathogen and in each type of laboratory need to be interpreted with caution as the data represent only those laboratories that reported numerical values.

Table 7: Number of faecal specimens tested for each bacterial pathogen, where an absolute value of one or greater was provided

Bacterial pathogen	Responses[a]	No. tested	Mean[b]	Median	Maximum	Minimum	Hospital Laboratories	Community Laboratories	Hospital/Community Laboratories	Public Health Laboratories
Salmonella spp.	26	109,332	4,205	1,267	48,143	78	16,053	76,923	15,659	697
Shigella spp.	26	107,981	4,153	1,267	48,143	17	16,053	76,123	15,659	146
Campylobacter spp.	26	108,016	4,154	1,267	48,143	47	16,060	76,123	15,659	174
Yersinia spp.	26	107,858	4,314	1,400	48,143	23	16,053	76,123	15,659	23
Listeria spp.	3	53	17	20	32	1	20	32	0	1
STEC incl. *E. coli* O157:H7[c]	18	9,422	523	221	2,500	7	4,525	4,694	160	43
Aeromonas spp.	14	15,670	1,119	726	4,013	3	11,763	1,410	2,497	0
Vibrio spp.	8	7,776	972	829	2,160	2	4,781	2,978	15	2
Plesiomonas spp.	13	20,416	1,570	1,133	4,013	409	14,083	4,524	1,809	0
Enterobacter sakazakii [c]	2	764	382	382	763	1	763	1	0	0
Bacillus spp.[c]	2	275	137	138	219	56	0	0	0	275
Clostridium spp.[c]	3	1,248	416	145	1,062	41	0	0	1,062	186
Staphylococcus aureus [c]	4	1,474	368	338	763	35	1,321	0	0	153

[a] Number of laboratories from 35 total responses providing a response for the pathogen (absolute value ≥1).

[b] Total number tested/number of responses.

[c] Not toxin testing.

There were several responses where information was given but it was not sufficient to be recorded in Table 7, i.e. an absolute value of one or greater was not recorded. This information (Table 8) provides some indication for why many participants could not provide these data or where testing was not undertaken for particular bacterial pathogens.

Table 8: Testing of faecal specimens for bacterial pathogens – non-numerical responses or data <1

Bacterial pathogen	No. responses for:		Other responses
	Nil, 0, <1	"Data not available"	
Salmonella spp.	0	0	-
Shigella spp.	0	0	-
Campylobacter spp.	0	0	-
Yersinia spp.	0	0	-
Listeria spp.	6	3	Very few, maybe one every 1-2 weeks; <5
STEC incl. *E. coli* O157:H7	0	4	Guess 20-30% of faeces
Aeromonas spp.	2	3	Guess 15-25 % faeces
Vibrio spp.	3	6	Guess 15-25 % faeces
Plesiomonas spp.	3	3	Guess 15-25 % faeces
E. sakazakii	6	0	1-2 per year
Bacillus spp.	5	0	-
Clostridium spp.	5	0	-
S. aureus	5	0	One or two per year

In summary, Salmonella, Campylobacter, Yersinia and Shigella are the bacterial pathogens for which faecal specimens are tested most frequently. Testing is markedly less common for STEC, Aeromonas, Vibrio and Plesiomonas, and very low for all others.

3.5.2 Testing criteria

Q13.2-Q25.2: Participants were asked to indicate the criteria for testing faecal samples for 13 specific bacterial pathogens.

Q26.2: Participants were asked to indicate the criteria for testing faecal samples for any other bacterial pathogen not specified in Q13-Q25.

The criteria used by the surveyed laboratories for testing a faecal specimen for each bacterial pathogen are summarised in Table 9, along with qualitative comments. Figure 5 illustrates the overall pattern of response to these questions.

Table 9: Criteria used in laboratories for testing faecal specimens for each bacterial pathogen

Criteria were as follows:

- Test routinely as part of enteric screen (= routine)
- Only test if specifically requested by referring doctor (= request)
- Laboratory has own criteria for testing (= own) – see below
- Not applicable, do not test for this organism (= N/A)

Bacterial pathogen	Total responses[a]	Responses to criteria			
		Routine	Request	Own	N/A
Salmonella spp.	34 (97%)	32	0	2	0
Shigella spp.	34 (97%)	32	0	2	0
Campylobacter spp.	35 (100%)	33	0	2	0
Yersinia spp.	34 (97%)	32	0	2	0
Listeria spp.	33 (94%)	1	22[b]	5	8
STEC (incl. *E. coli* O157:H7)	34 (97%)	7	13[b]	26	0
Aeromonas spp.	34 (97%)	19	5	8	5[c]
Vibrio spp.	34 (97%)	8[d]	19[b]	18	3[b]
Plesiomonas spp.	34 (97%)	19	2	5	9[e]
Enterobacter sakazakii	30 (86%)	2[g]	13[g]	0	19[b,f]
Bacillus spp.	33 (94%)	0	1[b]	2	30
Clostridium spp.	33 (94%)	0	4[b,h]	5	27
Staphylococcus aureus	33 (94%)	8[i]	4	3	18
Other enteric bacteria	2 (6%)	0	0	1	1

[a] Some laboratories selected more than one criterion, e.g. ticked 'request' and also gave their own criteria. The total responses is the number of laboratories, out of the 35 surveys received, that answered to one or more of the criteria and is not necessarily equivalent to the sum of 'responses to criteria'.

[b] One laboratory noted that they would refer the specimen to another laboratory.

[c] One laboratory noted that Aeromonas has been isolated during routine culture for *E. coli*.

[d] One laboratory noted that 'in 2005 any faeces with high WBC or clinical details suggestive (of Vibrio) were cultured specifically. Protocol changed in 2006 to specific culture only on request or clinicals suggested e.g. seafood ingestion. All clinical isolates of *Vibrio* have been detected on blood agar.

[e] One laboratory noted that Plesiomonas has been isolated from routine culture plates.

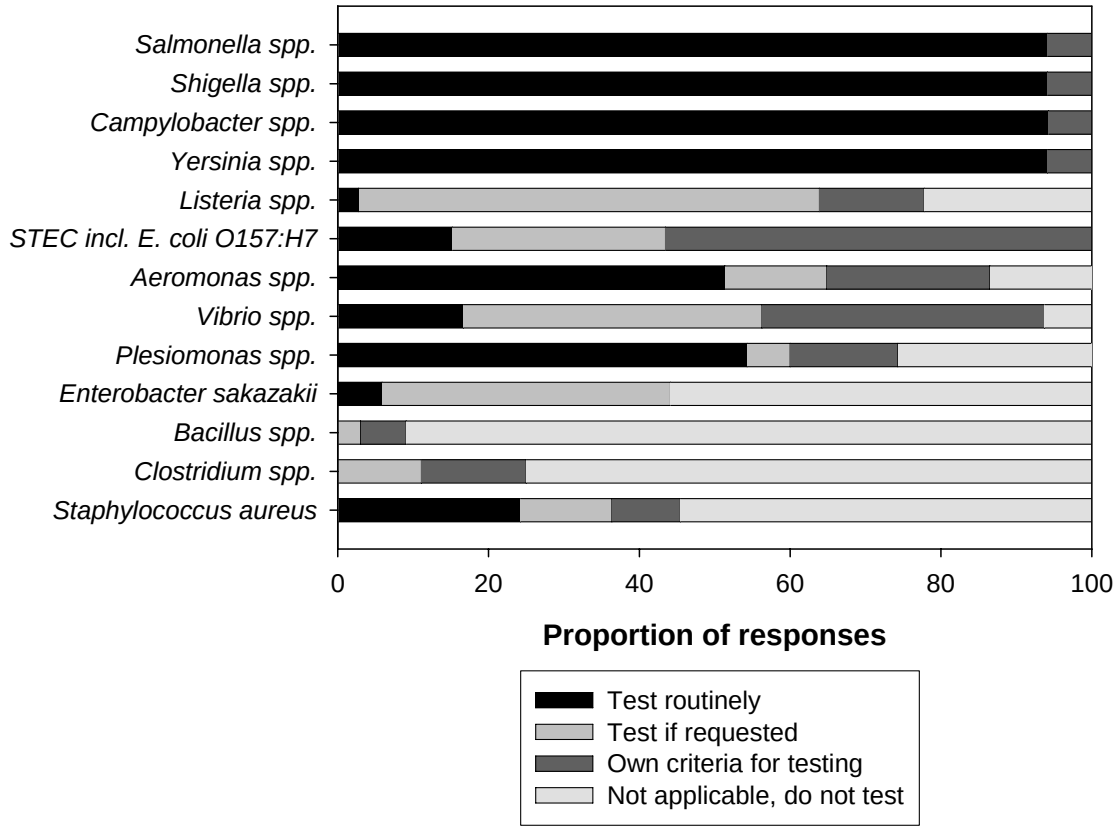
[f] One laboratory selected both 'routine' and 'N/A' but gave the comment 'will mention if isolated'. Given the information supplied on testing methods, this has been interpreted to mean that while this laboratory does not specifically test for *E. sakazakii*, it can be isolated from routine tests for other bacterial pathogens.

[g] Two laboratories noted that they would refer the specimen to another laboratory.

[h] One laboratory stated *Cl. difficile*, interpreted to mean that they would only test for *Clostridium difficile* if requested.

[i] One laboratory noted 'only noted if no normal faecal flora is present'.

Figure 5: Criteria used by laboratories for testing for bacterial pathogens in faecal specimens



'Own criteria' for testing

Where laboratories have indicated that they use their own criteria for testing for specific bacterial pathogens, and have specified that criteria, the responses were summarised as follows:

(i) *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp. and *Bacillus* spp:

The two Public Health laboratories indicated that testing for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp. and *Bacillus* spp depended on the clinical history, symptoms and condition of the specimen.

(ii) *Listeria* spp:

Five laboratories listed their own criteria for testing for Listeria. These were as follows:

- Pregnancy
- Clinical details
- Pregnant with diarrhoea
- Depends on clinical history
- Based on symptoms/onset/food history/risk factors/specimen condition

(iii) STEC incl. *E. coli* O157:H7:

Twenty-six laboratories listed their own criteria for testing for STEC. They are summarised as follows:

- Bloody diarrhoea/specimen (20 responses)
- Relevant clinical details e.g. HUS, thrombocytopaenia (9 responses)
- All children <12 years (12 responses)
- All children <10 years (3 responses)
- All children <5 years (2 responses)
- Red blood cells present (4 responses)
- Overseas travel (1 response)
- Indicated that had own criteria, but did not specify (2 responses)

(iv) *Aeromonas* spp:

Eight laboratories listed their own criteria for testing for Aeromonas. The criteria were:

- 'Liquid' or 'fluid' specimens (6 responses)
- Clinical details (3 responses)
- Testing of oxidase positive organisms (1 response)
- <12 yrs or bloody/liquid specimen, overseas travel (1 response)

(v) *Vibrio* spp:

Eighteen laboratories listed their own criteria for testing for Vibrio. They are summarised as follows:

- Liquid/fluid specimens (7 responses)
- Clinical details (7 responses)
- Seafood consumption (6 responses)
- Overseas travel (6 responses)
- RBCs present (1 response)

(vi) *Plesiomonas* spp:

Five laboratories reported having their own criteria for testing for Plesiomonas, although one did not specify the criteria. The criteria of the remaining four were:

- 'Fluid specimen' (3 responses, one also with 'clinical details')
- 'Testing of oxidase positive organisms' (1 response)

(vii) *Clostridium* spp:

Five laboratories listed their own criteria for testing for Clostridium. They were as follows:

- Clinical details (test for *C. difficile*)
- Clinical particulars indicate antibiotic treatment
- Would do toxin testing first
- Depends on clinical history
- Based on symptoms/onset/food history/risk factors/specimen condition

(viii) *Staphylococcus aureus*:

Three laboratories listed their own criteria for testing for *S. aureus*. They were as follows:

- 'Predominant organism'.
- Depends on clinical history

- Based on symptoms/onset/food history/risk factors/specimen condition

(iv) Other enteric bacteria:

Only one laboratory indicated that they had their own criteria for testing for enteric bacteria not specified in the previous questions. This laboratory tested for vancomycin-resistant *Enterococcus* spp., but did not specify the criteria.

3.5.3 Methods

Q13.3-Q25.3: Participants were asked for information on the method used for testing faecal samples for 13 specific bacterial pathogens.

Q26.3: Participants were asked for information on the method used for testing faecal samples for any other bacterial pathogen not specified in Q13-Q25.

The responses to these questions have been summarised for each bacterial pathogen.

Salmonella spp.

(i) Selective media:

A total of 34 laboratories provided information. Of these, 32 laboratories reported using Xylose Lysine Desoxycholate (XLD) agar, of which 10 did not list any other selective media. The other 22 laboratories combined XLD with other selective agars, usually just one other, but one laboratory used four additional agars. The number of laboratories using XLD with other agars is as follows:

- Hektoen Enteric Agar (15 responses)
- MacConkey Agar (9 responses)
- Brilliant Green Agar (2 responses)
- Bismuth Sulphite Agar (2 responses)

Of the remaining two laboratories, one reported using only Hektoen and the other MacConkey with Statens Serum Institut Agar.

(ii) Enrichment:

Used by 32 laboratories (several noted that they used selenite broth or selenite F broth).

(iii) Incubation:

Twenty-five laboratories reported an incubation temperature, and all but one gave temperatures between 35ºC and 37ºC (one also noting "or 42ºC"). The remaining laboratory incubated at room temperature.

(iv) Other methods:

One laboratory reported using an enzyme immunoassay (EIA) for screening Salmonella.

(v) Comments:

Hektoen Enteric, MacConkey, Brilliant Green and Bismuth Sulphite agars are all appropriate for isolating Salmonella and Statens Serum Institut Agar is used for setting up antimicrobial resistance testing. Enrichment in selenite broth is common practice for increasing the probability of detecting Salmonella. Salmonella grows well at all temperatures noted.

Shigella spp.

(i) Selective media:

A total of 33 laboratories provided information on selective media for isolating Shigella. These media, and their combinations, are very similar to those used for Salmonella, indicating that both bacteria are isolated by the same method. This was confirmed by one laboratory who commented "we look for Shigella off Salmonella cultures as well". In summary, the following media were reported as being used by laboratories:

- Xylose Lysine Desoxycholate Agar (29 responses)
- Hektoen Enteric Agar (16 responses)
- MacConkey Agar (11 responses)
- Brilliant Green Agar (2 responses)
- Bismuth Sulphite Agar (1 response)

(ii) Enrichment:

Used by 18 laboratories (several noted that they used selenite broth or selenite F broth).

(iii) Incubation:

Twenty laboratories reported an incubation temperature, all between 35ºC and 37ºC.

(iv) Other methods:

No responses.

(v) Comments:

Shigella will be enriched in Selenite broth (though not optimally) and can be isolated on Xylose Lysine Desoxycholate, Hektoen Enteric or MacConkey Agars, but grows poorly or not at all on Brilliant Green and Bismuth Sulphite Agar. The incubation temperatures are appropriate for this pathogen.

Campylobacter spp.

(i) Selective media:

A total of 33 laboratories reported the selective media used for isolating Campylobacter. Seventeen laboratories were not specific: 8 laboratories used Campylobacter blood-free agar, while 9 laboratories used Campylobacter agar. Four laboratories specifically reported using a blood free Campylobacter agar called CCDA or mCCDA. Three laboratories used a variation of Campylobacter blood-free agar called CAT, which differs by the antibiotics added to the blood-free agar base (Cefoperazone, Amphotericin B and Teicoplanin; mCCDA does not contain Teicoplanin

and the concentrations of Cefoperazone and Amphotericin B differ). Two laboratories reported using campylobacter-charcoal which may be either CAT or CCDA.

Six laboratories reported using a blood-based agar, of which three used Skirrows, two Exeter and the remaining laboratory reported the media only as Campylobacter isolation media (blood). One laboratory reported using blood free Campylobacter media (Prestons).

(ii) Enrichment:

No enrichment was reported by any of the laboratories.

(iii) Incubation:

Twenty-eight laboratories reported an incubation temperature of 42ºC. Of a further three responses, one laboratory incubated at 35ºC, another at 37ºC and a third at 37ºC and 42ºC. The atmospheric conditions were not requested, however 12 laboratories indicated that incubation was carried out under microaerophilic or CO_2 conditions.

(iv) Other methods:

One laboratory reported that PCR was used for identification of isolates.

(v) Comments:

It appears that CAT and CCDA/mCCDA are the most common agars used for Campylobacter isolation. CCDA and mCCDA favour isolation of *Campylobacter jejuni* and *Campylobacter coli* over other *Campylobacter* species. For this reason, CAT is preferred by some laboratories as it permits better recovery of other *Campylobacter* species, such as *C. upsaliensis*, when compared to mCCDA. Exeter is comparable with mCCDA. Skirrow's was commonly used in the 1980's, prior to the commercialisation of mCCDA.

Campylobacter jejuni and *Campylobacter coli* will grow at all temperatures listed, but optimally at 42ºC. However, some other species of Campylobacter do not grow at 42°C and will not be isolated. Microaerophilic or CO_2 conditions are required for the pathogen to grow on agar. PCR is used for isolate identification and is a more accurate alternative to biochemical tests such as hippurate and nalidixic acid resistance.

Yersinia spp.

(i) Selective media:

Thirty-four laboratories reported using a selective media for isolation of Yersinia. All used Yersinia isolation agar, which is also known as Cefsulodin-Irgasan Novobiocin (CIN) agar.

(ii) Enrichment:

Eight laboratories reported using enrichment for isolation of Yersinia. While not specifically requested, three laboratories noted that they enriched in selenite broth or selenite F broth, and two use Yersinia Selective Enrichment broth (also known as Ossmer broth).

(iii) Incubation:

The temperatures reported by the 29 laboratories responding to this question varied between 25ºC and 37ºC, and are summarised as follows:

- 25ºC to 28ºC (7 responses)
- 30ºC (13 responses)
- 35ºC to 37ºC (8 responses)
- Room temperature and 37ºC (1 response)

(iv) Other methods:

No responses.

(v) Comments:

CIN agar is appropriate for isolation and identification of Yersinia, though successful identification of Yersinia colonies from other microflora will depend on the experience of the laboratory worker. Enrichment would be necessary for specimens where Yersinia might be present in low numbers, and Yersinia selective enrichment broth helps to discourage background microflora. Selenite broth is not optimal for Yersinia. Yersinia will grow at all temperatures listed, however the pathogen can also grow under cool conditions and is a poor competitor with other microflora, so cooler incubation temperatures are better for discouraging outgrowth of background microflora and create more opportunity for Yersinia to be isolated. It has also been shown that incubation of Yersinia at temperatures above 30ºC can cause loss of a plasmid-based virulence factor, which is important for identification of virulent strains of Yersinia from avirulent strains.

Listeria spp.

(i) Selective media:

Fourteen laboratories listed the selective media used for isolation of Listeria. One reported simply "Listeria isolation media". This name is often used for Oxford agar, which was used by six laboratories. Three laboratories reported using PALCAM (two of these in association with Oxford agar), and three laboratories used CNA. All of these are blood-based agars that vary mostly in the antibiotics and sugars making up the final formulation. The remaining laboratory reported using "Aztreonam agar". Aztreonam is an antibiotic, but it has been used with many agar formulations, and no further information was provided.

(ii) Enrichment:

Five laboratories indicated that they used enrichment, with two noting that they enriched in "Listeria enrichment broth".

(iii) Incubation:

Of the ten laboratories recording an incubation temperature, seven incubated at 35-36ºC, one at 30ºC and another at 27ºC. The remaining response was 35ºC under CO_2 conditions (may have been confused with Campylobacter).

(iv) Other methods:

One laboratory also looked for "typical colonies on sheep blood agar".

(v) Comments:

Oxford and Palcam are the most appropriate agars to use. *Listeria monocytogenes* can tolerate nalidixic acid and conlinstin, so should grow on CNA, but this agar is more suited to gram positive cocci. Listeria are facultative anaerobes so will grow under CO_2, but not optimally. It will also grow at all temperatures listed, though the rate of growth will vary.

STEC incl. *E. coli* O157:H7

(i) Selective media:

Of the 32 responses for this question, 11 laboratories reported using Sorbitol MacConkey (SMAC) agar for isolation of STEC. The remaining 21 laboratories used a variation of SMAC that assists with the specific isolation of *E. coli* O157:H7 by the addition of a Cefixime-Tellurite supplement (CT-SMAC). Of those using CT-SMAC, two also used a blood-based agar called Enterohaemorrhagic *E. coli* (EHEC). One laboratory used MacConkey agar in addition to CT-SMAC and EHEC.

(ii) Enrichment:

Two laboratories indicated that they used an enrichment step for isolation of STEC.

(iii) Incubation:

Nineteen laboratories incubated between 35ºC and 37ºC. Another laboratory incubated at 42ºC, but noted that this was only for O157 enrichment. One other laboratory incubated at 35ºC and 42ºC. The two laboratories recording 42ºC as an incubation temperature were those who indicated that they carried out an enrichment step (above).

(iv) Other methods:

Two laboratories reported other methods of STEC detection. One used immunomagnetic separation (IMS) and PCR, and the other IMS with an enzyme immunoassay (EIA).

(v) Comments:

E. coli O157:H7 will grow on all agars listed, though CTSMAC is more selective than SMAC and MacConkey, and EHEC is non-selective. Successful identification of STEC from background microflora on these media will depend on the experience of the laboratory worker. STEC will grow at all temperatures listed. IMS helps to enhance the isolation of STEC from faeces, and PCR and EIA are isolate screening tools.

Aeromonas spp.

(i) Selective media:

Twenty-four laboratories recorded the media used for Aeromonas isolation, of which 12 used Aeromonas agar and one reported that they "commenced using selective media in 2006". Another laboratory reported that they found Yersinia agar to be useful, and two laboratories recorded using CIN agar. One of the laboratories using CIN agar also used blood agar, and a further seven laboratories also used non-selective blood agar either alone, or in one case with MacConkey agar. The remaining laboratory did not record a specific media for isolation of Aeromonas, but noted "will grow on most enteric media but most evident on CT-SMAC".

(ii) Enrichment:

No enrichment was reported by any of the laboratories.

(iii) Incubation:

Of the 14 responses to this question, eight laboratories incubated between 35ºC and 37ºC, five at 30ºC and one at 28ºC.

(iv) Other methods:

One laboratory, which did not provide any method information, noted that they "pick off routine plates".

(v) Comments:

Aeromonas should grow within the temperature range 28ºC to 37ºC. Aeromonas isolation agar is the most appropriate media to use, and the pathogen will also grow on blood agars.

Vibrio spp.

(i) Selective media:

Twenty-three of the 29 laboratories reported using Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar for isolation of Vibrio, and one recorded that they "commenced using selective media in 2006". Four laboratories used a non-selective blood agar, of which one combined this with TCBS and another with MacConkey agar. Of the remaining two laboratories, one used Hektoen and XLD agars and the other Aeromonas and Yersinia agar.

(ii) Enrichment:

Only one laboratory indicated that they had an enrichment step. This laboratory used TCBS as the selective agar.

(iii) Incubation:

All 14 of the respondents to this question incubated between 35ºC and 37ºC.

(iv) Other methods:

Two laboratories elected to respond to this question, both indicating that Vibrio are also recognised through the routine plating of faecal specimens on a non-selective blood agar.

(v) Comments:

TCBS and blood agar will grow Vibrio. The other agars mentioned may also allow Vibrio to grow sub-optimally and would require reasonable experience for identification of the Vibrio colonies (as would blood agar). The incubation temperatures are appropriate.

Plesiomonas spp.

(i) Selective media:

None of the 17 responses to this question listed any media that were specifically selective for Plesiomonas. All of the media listed are used were either non-selective (blood plates, 7 laboratories) or used for isolation of other bacterial pathogens. These selective media included XLD, Hektoen,

Aeromonas agar, Yersinia agar and MacConkey agar. Some comments from laboratories included "will grow on XLD, Hek (i.e. Hektoen Enteric Agar) but most likely picked up on Yersinia plate", "part of usual work up, no special media", "pick off routine plates" and "may find incidentally on plates incubated".

(ii) Enrichment:

No enrichment was reported by any of the laboratories.

(ii) Incubation:

Five laboratories indicated that they incubated between 35ºC and 37ºC.

(iv) Other methods:

No responses.

(v) Comments:

Plesiomonas is not a common pathogen and there is no particular selective agar commercially available. Isolation of Plesiomonas will depend on the skill of a laboratory worker in identifying likely positive colonies on the range of agars listed.

Enterobacter sakazakii

(i) Selective media:

Only four laboratories listed the media used for *E. sakazakii*, three of which recorded MacConkey agar and the fourth Chromocult agar. A fifth laboratory indicated that *E. sakazakii* were picked off routine plates.

(ii) Enrichment:

No enrichment was reported by any of the laboratories.

(iii) Incubation:

Three laboratories indicated that they incubated between 35ºC and 37ºC.

(iv) Other methods:

No responses.

(v) Comments:

The Chromocult agar is an expensive media designed specifically for *E. sakazakii*. Any media capable of growing *E. coli* should also grow *E. sakazakii* (see above), and 35ºC to 37ºC is an appropriate incubation temperature.

Bacillus spp.

Only two laboratories indicated that they cultured Bacillus and these were the only laboratories to provide any information on the method. Both laboratories used MYP agar (no enrichment) and incubated at 30ºC. MYP is an appropriate agar to use for isolation and identification of Bacillus, and 30ºC is the optimum growth temperature for this pathogen.

Clostridium spp.

(i) Selective media:

Only four laboratories listed the media used for Clostridium, two of which recorded Tryptose-Sulfite-Cycloserine (TSC) Agar. The other two used *Clostridium difficile* agar (CCFA), and one of these laboratories also used Fastidious Anaerobic Agar (FAA) after an alcohol shock for 30 min.

(ii) Enrichment:

No enrichment was reported by any of the laboratories.

(iii) Incubation:

Three laboratories indicated that they incubated between 35ºC and 37ºC.

(iv) Other methods:

No responses.

(v) Comments:

TSC agar is most appropriate for *Clostridium* spp., as CCFA will inhibit *C. perfringens*. FAA will grow *C. perfringens*. The agars must be incubated anaerobically; this information was not requested from the laboratories, and none of the respondents noted anaerobic incubation. The optimum temperature for growth is between 35ºC and 37ºC.

Staphylococcus aureus

(i) Selective media:

The ten laboratories that responded to this question listed variable media. Four used a sheep blood agar plate, and two used CNA, one combining CNA with mannitol salt agar. A further two laboratories used an aztreonam-supplemented agar, one also using Baird Parker agar, and the remaining two laboratories used Baird Parker agar alone.

(ii) Enrichment:

One laboratory indicated that they enriched in a salt broth.

(iii) Incubation:

Five responses, all 35ºC.

(iv) Other methods:

One laboratory reported also using Staphylax (*c.f.* Staphylex or Flucloxacillin, an antibiotic) and coagulase tests.

(v) Comments:

Blood agar, CNA or CNA/mannitol salt agar will all growth *S. aureus* well, but Baird Parker is the better selective agar as the pathogen is better recognised. Staphylax is used for confirmation of isolates.

Other enteric bacteria

The laboratory that reported isolating for Vancomycin resistant *Enterococcus* spp. uses Aztreonam agar for this purpose. No additional information was provided.

3.6 Viral Pathogens

Section G asked participants to provide information on the number of faecal specimens tested during 2005 for enteric viruses, and for each virus what criteria is used to decide if that virus should be tested for and the methods used to test the specimens.

3.6.1 Number tested

Q27.1-Q29.1: Participants were asked to provide the number of faecal samples tested in 2005 for three specific enteric viruses.

Q30.1: Participants were asked to provide the number of faecal samples tested in 2005 for any other enteric viruses not specified in Q27-Q29, and to specify that enteric virus.

Where laboratories provided data on rotavirus and norovirus testing with an absolute value of one or greater, these data were analysed and the results presented in Table 10. No laboratories tested any specimens for Hepatitis A virus. The data from the NRL have been incorporated in Table 10.

The numbers of samples tested for each individual pathogen and in each type of laboratory need to be interpreted with caution as the data represent only those laboratories that reported numerical values.

Table 10: Number of faecal specimens tested for rotavirus and norovirus where an absolute value of one or greater was recorded

Viral pathogen	Responses[1]	No. tested	Mean	Median	Max	Min	Hospital Laboratories	Community Laboratories	Hospital/Community Laboratories	Public Health Laboratories and NRL
Rotavirus	25 (71%)	23,522	941	230	9,686	50	6,732	15,016	1,774	0
Norovirus[2]	3 (8%)	673	224	207	356	110	110	0	0	563

[1] Number of laboratories providing a response for the pathogen, where any referral has not been indicated (absolute value ≥1).

[2] Data on norovirus testing provided by the NRL have been incorporated with data from two laboratories that provided responses to the norovirus questions. The percentage response is from 36 laboratories.

Seven laboratories recorded that they tested for 'other enteric viruses', of which two listed more than one virus. Four laboratories specified adenoviruses and three the more generic term enteroviruses (which may or may not include viruses responsible for gastrointestinal illness). Of the 51 types of adenovirus, types 40 and 41 are known to cause gastrointestinal illness, mainly in children, so these are likely to be the specific types tested for. One laboratory also specified poliovirus 1,2,3 and coxsackie A and B, though the potential neurological and respiratory effects of these agents are more important clinically. The number of specimens tested was only provided by four laboratories for adenoviruses; a total of 1,648 (median 321).

3.6.2 Testing criteria

Q27.2-Q29.2: Participants were asked to indicate the criteria for testing faecal samples for three specific enteric viruses.

Q30.2: Participants were asked to indicate the criteria for testing faecal samples for any other enteric virus not specified in Q27-Q29.

Thirty-one laboratories selected 'not applicable' for Hepatitis A virus, and the remaining four laboratories recorded no response for this virus, so it is omitted from the analysis. Hepatitis A virus does not cause gastrointestinal illness. The criteria used for testing faecal specimens for rotavirus and norovirus are presented in Table 11.

Table 11: Criteria used in laboratories for testing faecal specimens for rotavirus and norovirus

Criteria were as follows:

- Test routinely as part of enteric screen (= routine)
- Only test if specifically requested by referring doctor (= request)
- Laboratory has own criteria for testing (= own) – see below
- Not applicable, do not test for this organism (= N/A)

Viral pathogen	Total responses*	Responses to criteria			
		Routine	Request	Own	N/A
Rotavirus	33 (94%)	0	24	26	3
Norovirus	32 (91%)	0	2	2	0

* Many laboratories selected more than one criterion, e.g. ticked 'request' and also gave their own criteria. The total responses is the number of laboratories out of the 35 surveys received that answered any of the criteria and is not necessarily equivalent to the sum of 'responses to criteria'.

'Own criteria' for testing

Where laboratories have indicated that they use their own criteria for testing for specific viral pathogens, and have specified the criteria, the responses are summarised as follows:

(i) Rotavirus:

Twenty-five laboratories listed their own criteria for testing for rotavirus, some listing more than one criterion. They are summarised as follows:

- Children (2 responses), or as defined by age:
    - <2 years old (1 response)
    - <3 years old (3 responses)
    - <4 years old (1 response)
    - <5 years old (15 responses)

    - <6 years old (1 response)
    - <7 years old (1 response)
    - <12 years old (1 response)
- Elderly or rest home patients, or people >65 years (3 responses)
- Fluid specimen (1 response)
- Clinical details (1 response)

(ii) Norovirus:

Two laboratories listed their own criteria for testing for norovirus, "if part of an outbreak" (and the testing is referred to another laboratory) or "Based on symptoms/onset/food history/risk factors/sample condition".

(iii) Other enteric viruses:

- Enterovirus: Tested by request (3 responses) or if clinical details are relevant (1 response).
- Adenovirus: Tested if patient <5 years old (3 responses), by request (2 responses) or performed with any rotavirus test (1 response).

3.6.3 Methods

Q27.3-Q29.3: Participants were asked for information on the method used for testing faecal samples for three specific enteric viruses.

Q30.3: Participants were asked for information on the method used for testing faecal samples for any other enteric virus not specified in Q27-Q29.

Rotavirus

Of the 26 laboratories responding to this question, eight reported using Latex agglutination, four Enzyme Immunoassay (EIA) and the remaining 14 an 'other' method, specifically an Immunochromatographic (ICT) strip. PCR and culture were not selected.

Norovirus

Five laboratories responded to this question, of which three indicated that the laboratory specimens were referred to ESR for analysis. Of the remaining two laboratories, both used PCR.

Other enteric viruses:

The methods used for testing for the other enteric viruses listed were as follows:

- Enterovirus: EIA (1 response), culture (3 responses).
- Adenovirus: ICT (3 responses), latex (2 responses).

Comments:

Rotavirus is best detected by non-culture methods such as latex agglutination and EIA or ICT strips. Some ICT strips have also been designed to also detect adenovirus types 40 and 41 in the same assay. While non-gastrointestinal adenoviruses can be cultured, adenovirus types 40 and 41 cannot be cultured easily and are best detected by ICT, PCR or latex agglutination. Specific norovirus EIA assays are available, but PCR is the most sensitive assay currently available.

Sapovirus and astrovirus are other viral agents capable of causing AGI, but which are not often considered for testing.

3.7 Parasites and Protozoa

Section H asked participants to provide information on the number of faecal specimens tested during 2005 for parasites and protozoa, and for each parasite or protozoa, what criteria is used to decide if it should be tested for and the methods used to test the specimens.

3.7.1 Number tested

Q31.1-Q33.1: Participants were asked to provide the number of faecal samples tested in 2005 for three specific protozoa/parasites.

Q34.1: Participants were asked to provide the number of faecal samples tested in 2005 for any other protozoa/parasites not specified in Q31-Q33, and to specify that protozoa/parasite.

Where laboratories provided data for the testing of parasites and protozoa where an absolute value of one or greater was recorded, these data were analysed and the results presented in Table 12.The numbers of samples tested for each individual pathogen and in each type of laboratory need to be interpreted with caution as the data represent only those laboratories that reported numerical values.

Fifteen laboratories recorded testing of 'other protozoa/parasites', of which 8 specified more than one species. Some responses were generalised, e.g. 'all others', 'flagellates' or 'protozoa'. The specific species listed were as follows:

Protozoans:

- Microsporidia
- Entamoeba (e.g. *Entamoeba histolytica*, *Entamoeba hartmanii*, *Entamoeba coli*)
- Dientamoeba (e.g. *Dientamoeba fragilis*)
- *Endolimax nana*
- *Iodamoeba butschlii*
- Isospora
- Cyclospora
- *Blastocystis hominis*
- Chilomastix

Parasites:

- Enterobius (pinworm)
- Ascaris (roundworm)
- *Strongyloides stercolaris* (roundworm)

Ten laboratories provided information on the number of specimens tested for these protozoans/parasites, but the values were not always attributable to one particular species. The total number of faecal specimens tested for protozoa/parasites other than ova, Giardia or Cryptosporidium was 5,385 (mean 539; median 226; max/min 2,562/20). The Entamoeba appear to be most frequently analysed (e.g. one laboratory analysed 1,100 specimens for Entamoeba, and another 2,562 for Entamoeba spp., *D. fragilis* and/or *B. hominis*).

Table 12: Number of faecal specimens tested for each protozoa or parasite where an absolute value of one or greater was recorded

Protozoa/parasite	Responses*	No. tested	Mean	Median	Max	Min	Hospital Laboratories	Community Laboratories	Hospital/Community Laboratories	Public Health Laboratories
Ova	18 (51%)	50,802	2,822	1,111	24,581	31	5,625	42,515	2,662	0
Giardia	24 (69%)	71,389	2,975	1,031	32,370	36	8,955	52,748	9,650	36
Cryptosporidium	22 (63%)	67,458	3,066	849	32,370	36	6,188	51,588	9,646	36

* Number of laboratories providing a response for the pathogen (absolute value ≥1

3.7.2 Testing criteria

Q31.2-Q33.2: Participants were asked to indicate the criteria for testing faecal samples for three specific protozoa/parasites.

Q34.2: Participants were asked to indicate the criteria for testing faecal samples for any other protozoa/parasites not specified in Q31-Q33.

The criteria used for testing faecal specimens for enteric protozoa and parasites are presented in Table 13.

Table 13: Criteria used in laboratories for testing faecal specimens for enteric protozoa and parasites

Criteria were as follows:

- Test routinely as part of enteric screen (= routine)
- Only test if specifically requested by referring doctor (= request)
- Laboratory has own criteria for testing (= own) – see below
- Not applicable, do not test for this organism (= N/A)

Protozoa/parasite	Total responses*	Responses to criteria			
		Routine	Request	Own	N/A
Ova	34 (97%)	2	22	11	7
Giardia	33 (94%)	6	24	9	1
Cryptosporidium	32 (91%)	6	22	11	1
Others	25 (71%)	3	15	13	1

* Many laboratories selected more than one criterion, e.g. ticked 'request' and also gave their own criteria. The total responses is the number of laboratories, out of the 35 surveys received, that answered any of the criteria and is not necessarily equivalent to the sum of 'responses to criteria'.

'Own criteria' for testing

Where laboratories have indicated that they use their own criteria for testing for specific enteric protozoa or parasites, and have specified the criteria, the responses are summarised as follows:

(i) Ova:

Eleven laboratories listed their own criteria for testing for Ova, some listing more than one criterion. They are summarised as follows:

- Relevant clinical details (5 responses).
- History of overseas travel (7 responses).
- All faecal specimens have a wet film examination; if something seen in wet film (2 responses).

(ii) Giardia:

Nine laboratories listed their own criteria for testing for Giardia, some listing more than one criterion. They are summarised as follows:

- Relevant clinical details (5 responses).
- Children (3 responses).
- All faecal specimens have a wet film examination; if something seen in wet film (2 responses).

- Waterborne illness (1 response).
- Fluid specimen (1 response).

(iii) Cryptosporidium:

Eleven laboratories listed their own criteria for testing for Cryptosporidium, some listing more than one criterion. They are summarised as follows:

- Relevant clinical details (5 responses).
- Children (5 responses).
- All faecal specimens have a wet film examination; if something seen in wet film (2 responses).
- Fluid specimen (2 responses).
- Reported with Giardia test.

(iv) Other protozoa/parasites:

Thirteen laboratories listed their own criteria for testing for other protozoa/parasites, some listing more than one criterion. They are summarised as follows:

- Relevant clinical details (5 responses).
- Age (1 response).
- Overseas travel (7 responses).
- Immunosuppressed/immunocompromised patient, e.g. AIDS (6 responses).
- All faecal specimens have a wet film examination; if something seen in wet film (3 responses).

3.7.3 Methods

Q31.3-Q33.3: Participants were asked for information on the method used for testing faecal samples for three specific protozoa/parasites.

Q34.3: Participants were asked for information on the method used for testing faecal samples for any other protozoa/parasites not specified in Q31-Q33.

Ova

Twenty-five laboratories responded to this question, many selecting more than one method. Of the specifically-listed methods, microscopy was most commonly-selected (19 responses), followed by PVA/SAF (16 responses), EIA (3 responses) and Antigen (1 response). Immunofluorescence was not selected by any laboratory.

Sixteen laboratories also listed at least one 'other method'. These are summarised as follows:

- 'Concentration' (5 responses), Ethylacetate sedimentation concentration (6 responses), Formalin concentration microscopy (2 responses), Formal saline concentrate (1 response) and MIF concentration (a commercial kit using formaldehyde and thimerosal; 1 response).
- Blood plate (for Strongyloides) (1 response).
- Kinyoun acid fast (1 response).

Giardia

Thirty-two laboratories responded to this question, many selecting more than one method. Of the specifically-listed methods, EIA was selected by 15 laboratories, 13 selected Antigen, 11 Microscopy, 9 Immunofluorescence (two noting that this was DFA or Direct Fluorescent Antigen Assay) and 6 chose PVA/SAF.

Seven laboratories also listed at least one 'other method'. These are summarised as follows:

- ‘Concentration’ (1 response), Formalin concentration (1 response), Ethylacetate sedimentation concentration (2 responses).
- Immunochromatographic strip (2 responses).
- Rapid haemochromogenic strip (1 response).
- Iron haematoxylin (1 response).

Cryptosporidium

Thirty-two laboratories responded to this question, many selecting more than one method. Of the specifically-listed methods, EIA was selected by 14 laboratories, 14 selected Antigen, 5 Microscopy, 11 Immunofluorescence (three noting that this was DFA) and 2 chose PVA/SAF.

Eight laboratories also listed at least one ‘other method’. These are summarised as follows:
- Concentration (1 response).
- Modified ZN stain (3 responses).
- Modified acid-fast stain (2 responses).
- Iron haematoxylin (1 response).
- Immunochromatographic strip (1 response).

Other protozoa/parasites

Twenty-two laboratories responded to this question, many selecting more than one method. The two most commonly selected methods for testing other parasites were Microscopy (13 responses) and PVA/SAF (15 responses). Immunofluorescence and EIA were each selected once.

Sixteen laboratories also listed at least one ‘other method’. These are summarised as follows:
- ‘Concentration’ (5 responses), Formalin/ethyl acetate concentration (2 responses) and MIF concentration (1 response).
- Trichrome (6 responses).
- Iron haematoxylin (2 responses).
- Modified ZN stain (1 response).
- Kinyoun staining (1 response).
- UV (for Cyclospora).

Comments:
The various concentration methods are all slightly different approaches to enable the parasites or protozoa to be visualised under a microscope. Stains can be used to increase the possibility of detection. The success of all these methods relies on the competence of the microscopist in recognising the parasites and protozoa under the field of view. The immunochromatographic and haemochromogenic strips are very similar and detect antigen from a faecal extract.

3.8 Toxins

Section I asked participants to provide information on the number of faecal specimens tested during 2005 for particular bacterial toxins, and for each toxin, what criteria are used to decide if it should be tested.

3.8.1 Number tested and testing criteria

Q35.1-Q39.1: Participants were asked to provide the number of faecal samples tested in 2005 for five specific bacterial toxins.

Q40.1: Participants were asked to provide the number of faecal samples tested in 2005 for any other toxins not specified in Q35-Q39, and to specify that toxin.

Q35.2-Q39.2: Participants were asked to indicate the criteria for testing faecal samples for five specific bacterial toxins.

Q40.2: Participants were asked to indicate the criteria for testing faecal samples for any other toxins not specified in Q35-Q39.

For all toxins other than that of *Clostridium difficile*, the responses to these questions were low and the specimen data and criteria for testing information are summarised together, as follows.

Staphylococcus aureus, Bacillus cereus and Clostridium perfringens

Only one laboratory provided data on the number of faecal specimens tested for *S. aureus* toxin (25 specimens), *B. cereus* toxin (4 specimens) and *C. perfringens* toxin (1 specimen). A second laboratory indicated that no specimens were tested for any of the three toxins. The number of laboratories indicating that they did not test for a toxin by selecting 'Not applicable, do not test for this toxin' as their criteria for testing was 31 for *S. aureus* toxin and 32 for *B. cereus* and *C. perfringens* toxins. The one laboratory that tested for these toxins during 2005 uses their own criteria for testing, and for all three toxins this was noted as "depends on clinical history and culture findings". Another laboratory also noted that they use their own criteria for testing for *S. aureus* toxin – if it is the "predominant organism" – however this laboratory did not provide any further information such as the number of specimens tested.

Clostridium difficile

Seventeen laboratories provided data on the number of faecal specimens tested during 2005 for *C. difficile* toxin. The total was 14,752, with 12,720 tested by hospital laboratories, 346 by community laboratories, and 1,686 by hospital/community laboratories. If it is assumed that samples tested for *C. difficile* are not often also tested for other bacterial pathogens, then samples tested for *C. difficile* toxin apparently make up the majority of the difference between samples tested for bacterial pathogens (16,053; Table 7) and the total samples (32,255; Table 4) tested by hospital laboratories. The mean specimen number per laboratory was 868, and the median 282 (maximum 3,380; minimum 45). Eleven laboratories indicated that they did not test for this toxin by selecting 'Not applicable, do not test for this toxin' as their criteria for testing. Of the other criteria for testing available for selection, 20 laboratories would test by request and 11 listed at least one of their own criteria for testing. The laboratory criteria for testing are summarised as follows:

- >3 days in hospital (7 responses)
- Clinical details, particularly past antibiotic use (8 responses)
- Liquid specimens (1 response)

STEC

Only two laboratories provided data on the number of faecal specimens tested during 2005 for STEC toxin. One laboratory was the only facility that routinely tested for STEC and analysed 2,106 specimens for STEC toxin in 2005. The other value (20) was from a laboratory that tested upon request. One further laboratory indicated that they tested by request, but did not provide any

specimen numbers for 2005. Several laboratories noted that they referred STEC-positive specimens on to other laboratories for toxin testing, and accordingly, 25 laboratories indicated that they did not test for this toxin by selecting 'Not applicable, do not test for this toxin' as their criteria for testing. Four laboratories had their own criteria for testing for STEC toxin, and of these three described their specific criteria, which was bloody diarrhoea and/or children under 12 years. Two laboratories specified the kit they used to test for STEC toxin, both being the Remel ProSpecT® Shiga Toxin *E. coli* (STEC) Microplate Assay.

3.9 Specimen Storage and Further Testing

3.9.1 Specimen storage

Q41: Are samples stored pending further testing requests?
If yes, how are they stored? If yes, for how long are they stored?

All laboratories answered Q41. Four (11%) of the laboratories do not store specimens pending further testing and subsequently did not provide storage information. The remaining 31 (89%) of laboratories did store specimens, under the following conditions summarised in Table 14.

Table 14: Storage time and conditions for faecal specimens after initial testing

Storage	**Number of laboratories using conditions**		
Time	Room temperature/ on bench	Refrigerated/ 4°C	Total
24 hours	0	1	1
3 days	0	5	5
4 days	0	2	2
1 week	3	16	19
2 weeks	1	1	2
1 month	0	1	1
2 months	0	1	1
Total	4	27	31

3.9.2 Non-detection of pathogens

Q42: In 2005, for how many samples that you tested (i.e. excluding discarded samples) was no pathogen identified?
Responses: 29/35 (83%)

Seventeen laboratories were able to provide figures for the number of specimens for which no pathogen was identified. These values were compared with the number of specimens received for testing by these laboratories to calculate the proportion of non-detection (Table 15). The number of specimens discarded prior to testing was not included in the analysis, as most laboratories did not have access to this information (Section 3.4.4), but number discarded appears to be small.

Table 15: Analysis of data from 17 laboratories on the number of tested faecal specimens for which no pathogen was identified, and the statistics of these non-detection specimens as a percentage of the total specimens received per laboratory

Factor	No. non-detection specimens	% of specimens received*
Total specimens	38,972	67.7
Mean specimens	2,292	76.5
Median specimens	1,964	80.0
Maximum value	11,422	91.2
Minimum value	65	17.3

* The value for 'total specimens' represents the total non-detection specimens as a percentage of total specimens received at the 17 laboratories. The other tabulated statistics are produced from a dataset of percentage non-detection from all 17 laboratories, e.g. the average (mean) percentage of non-detection is 76.5% and for one laboratory 91.2% were non-detection specimens (maximum).

A further four laboratories provided qualitative data or estimates that supports the analysis in Table 15. Their responses to Q42 were as follows:

- About 80% by culture, 85-90% for rotavirus, 95% for parasites. Total completely negative for all tests is unknown, probably 60-75%.
- Most.
- 84% negative for tested pathogens. This could not be very accurately determined…..
- Numbers not available - estimate 75% (using best data available).

One laboratory noted that a large number of specimens tested "were 'clearance' specimens from cases or contacts of notified illness. These specimens are usually tested for a single pathogen and by 'definition' have a high probability of being negative". This laboratory was able to provide data to show that no pathogen was detected in 83% of the 'clearance' specimens analysed in their laboratory.

The remaining eight laboratories indicated that they were unable to provide data on the number of non-detection specimens with comments such as "don't know", "unknown" and "data not available".

Of the specimens tested by the NRL, norovirus was not detected in 58.1%. Typical non-detection in this laboratory results from specimens being screened for norovirus during an outbreak, of which a portion will be negative and a further portion will not be tested once the outbreak is confirmed as norovirus, and where the causative agent is something other than norovirus.

3.9.2.1 Positivity rate for community faecal samples

Of the approximately 150,000 samples obtained from non-hospitalised patients (Table 4), the percentage of samples in which no pathogen was identified was reported (or could be estimated) by 18 community or "hospital & community" laboratories (excluding the Public Health Laboratories). Using reported proportions of sample sources (Question 5) the number of samples from non-hospitalised people tested by these 18 laboratories was calculated as approximately 59,000. Applying the reported percentages in which no pathogens were detected for each laboratory allowed the calculation that approximately 47,000 of these 59,000 samples (80%) had no pathogen identified. Calculating a 20% positivity rate from the sample provision rate of 0.02 – 0.06 samples per person per year suggests that the positivity rate in samples from non-hospitalised people is approximately 0.004 to 0.012 faecal samples per person per year. The positivity rate using the

sample provision rate from the Waikato would be higher, at 0.02 positive faecal samples per person per year.

This positivity rate of 20% for faecal samples from people in the community is consistent with overseas results. Community based studies in the Netherlands and UK found bacterial positivity rates of 16% and 19.5% respectively (de Wit *et al.*, 2001, Wheeler *et al.*, 1999), while the UK overall positivity rate was 24% (includes samples positive for viruses and parasites). A Canadian study in 2001 found that a pathogen (bacteria, parasite, *C. difficile,* or virus) was identified in 29.4% of stool samples (Flint, 2002).

3.9.3 Further testing

Q43: If no pathogen is identified from a sample, is further testing sometimes performed?
If yes, under what circumstances would further testing occur?
If yes, approximately, how many samples had further testing performed in 2005 after initial testing was all negative?
Responses: 33/35 (94%)

If no pathogen is identified from a faecal specimen, 21 laboratories do not perform further tests. Twelve laboratories do perform other tests, and all described at least one circumstance under which the additional testing would occur, summarised as follows:

- At the request of a doctor/clinician (7 responses).
- Following discussion with Public Health Units and consultants and/or if clinical data/information indicates it is warranted (3 responses).
- If suspicious structures seen on wet preparation/microscopy (e.g. white blood cells, red blood cells, mucus) (3 responses).
- Faecal specimen from a child referred for enteropathogenic *E. coli* testing if no pathogens detected in repeat specimens (1 response).
- *C. difficile* on post-antibiotic patients (1 response).
- If suspect rotavirus (1 response).

None of the laboratories were able to provide data on non-detection specimens that were tested further. Comments provided by six laboratories suggest that the specimen number is low (e.g. “very few”, “not many”, “<1%”, “<5%”), and another two laboratories gave estimates of “10-20 samples” and “40-50, usually infants”.

3.9.4 Specimen referral

Q44: Does your laboratory refer samples to either of ESR’s Public Health laboratories (PHL) in Auckland and Christchurch for additional testing?
If yes, what are the criteria?
Q45: Does your laboratory refer faecal samples to any of ESR’s virology laboratories in Wellington for additional testing?
If yes, please specify for which viruses this is done.
Q46: Which bacterial isolates do you send to ESR’s Enteric Reference Laboratory (ERL) in Wellington for further typing?

PHL referrals

Response: 32/33 (97%) (PHL responses are excluded)

Twenty-four laboratories do not refer specimens to the ESR PHL's for additional testing. Of the 8 laboratories that do, the criteria for referral are summarised as follows:

- Confirmation of an isolate and/or further identification, e.g. serotyping, parasitology (5 responses).
- Tests not performed on-site, e.g. toxin testing, norovirus, parasitology (4 responses).
- Isolates requested by ESR (1 response).
- Organisms of public health interest (1 response).

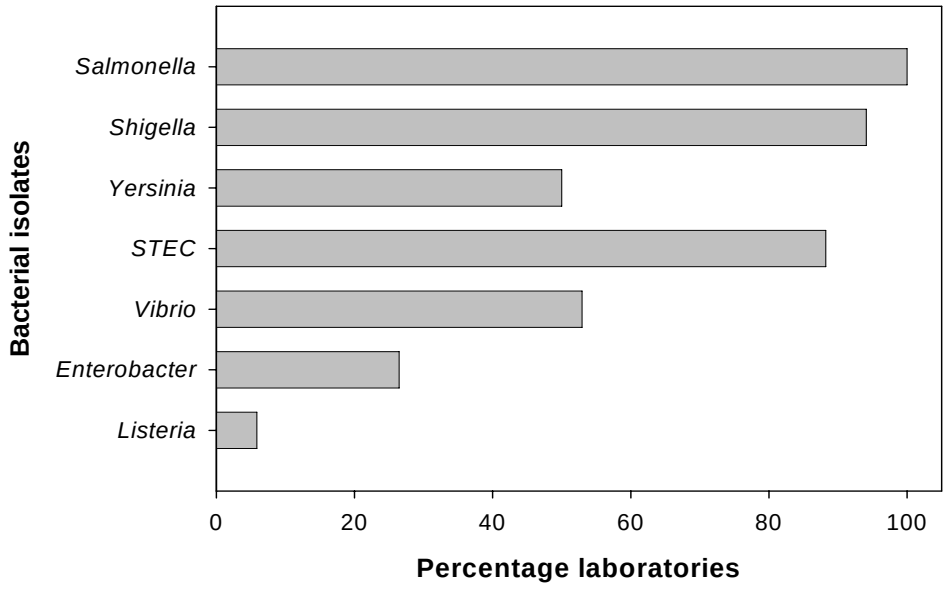
Virology referrals

Response: 33/35 (94%)
Nine laboratories do not refer specimens to ESR's virology laboratories for additional testing. Of the 24 laboratories that do, 21 refer for norovirus testing, of which three refer only if there is a suspected norovirus outbreak. One laboratory also refers specimens for adenovirus testing. Of the remaining three laboratories, one noted that they refer for tests not performed by their laboratory and the others did not provide any criteria.

ERL referrals

Response: 35/35 (100%)
All of the laboratories selected at least one of the specified isolates for referral to the ESR ERL, with the exception of one laboratory that referred all isolates to another laboratory, who would then refer isolates to ESR as required. These data are summarised in Figure 6 as a percentage of 34 laboratories, and includes two laboratories that indicated they refer isolates of *Listeria* or *L. monocytogenes* to the ERL. No other isolates were specified.

Figure 6: Percentage of laboratories (n=34) that refer specified bacterial isolates to the ESR ERL



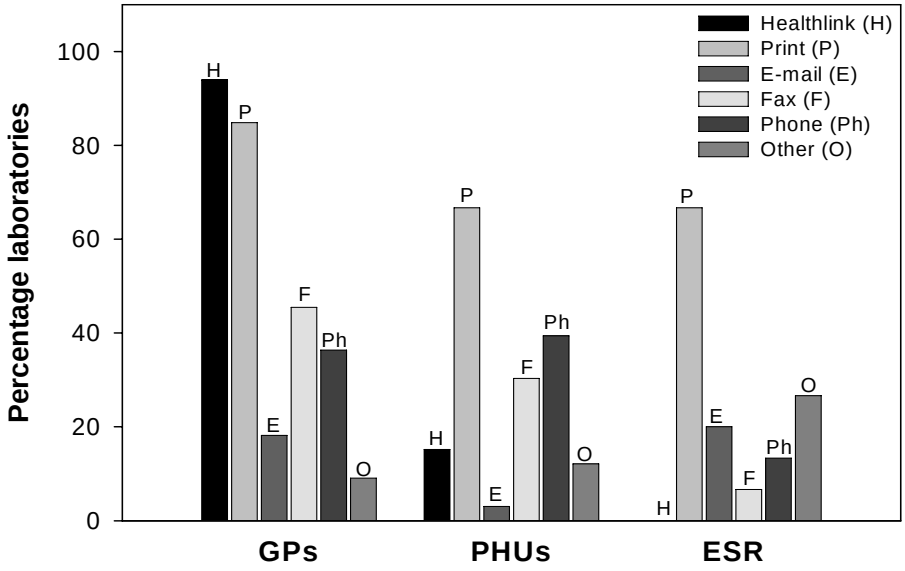
3.10 Reporting of Results

3.10.1 Typical reporting procedures

Q47: Laboratories were asked how results of faecal testing are usually sent to General Practitioners, Public Health Units/Medical Officer of Health and ESR.

Of all 35 laboratories, 33 (94%) selected at least one reporting option for GPs and for PHUs, and 15 (43%) selected at least one reporting option for ESR. Many selected more than one option. The results are summarised in Figure 7, with the proportions based on these response data.

Figure 7: Methods used by laboratories for reporting to General Practitioners (n=33), Public Health Units (n=33) or ESR (n=15)



The 'other' methods of reporting are summarised as follows:

- GPs (3 responses): Electronic reporting systems such as Éclair/Concerto, HL7
- PHUs (4 responses): Notification done by GP surgery, electronic reporting, don't know (how reporting done).
- ESR (4 responses): Information sent with request form and isolate.

The preference for the computer system Healthlink and print communication to GPs was common to both hospital and community (and hospital/community) labs. The only difference between these laboratory types in terms of communicating with GPs was that email was only used by community and hospital/community laboratories.

Healthlink was not used by hospital laboratories to communicate with PHUs.

3.10.2 Personal contacts

Q48: Are there any circumstances where someone from your laboratory would contact a faecal sample test requestor directly by phone? If yes, please specify.
Response: 35/35 (100%)

Q49: Are there any circumstances where someone from your laboratory would contact a Medical Officer of Health of Public Health Unit directly by phone regarding faecal sample test results? If yes, please specify.
Response: 34/35 (97%)

Four laboratories would not contact a test requestor specifically by phone, and eight laboratories would not contact a MOH or PHU directly by phone regarding faecal specimen test results. For the remaining laboratories that would use the telephone, the circumstances are summarised as follows:

Would contact a test requestor by phone if:

- Requests are:
  - Unclear or nothing has been requested (9 responses).
  - Unusual or do not fit with clinical details (4 responses)
  - From a GP asking for norovirus (attracts patient charge if no PHU involved) (1 response).
- More information is required on clinical details (14 responses)
- Specimen is inadequate or mislabelled (3 responses)
- Result reporting:
  - Positive results for pathogens of public health significance, e.g. *E. coli* O157, norovirus (6 responses).
  - Positive results for any pathogen (7 responses).
  - Result is urgent (1 response).
  - An outbreak is suspected (1 response).
- Request to do further testing (including toxin or parasite testing) (4 responses).

Would contact a MOH or PHU by phone if:

- Positive results for pathogens of public health significance, e.g. *E. coli* O157, *Vibrio cholerae*, *Salmonella typhi* (10 responses).
- Suspected or confirmed outbreak (3 responses).
- If requested to phone through result (7 responses).
- Notifiable pathogen isolated (6 responses).
- When rest homes or GPs request norovirus (1 response).
- Contacted as part of routine (2 responses).

4 COMPARISON WITH CAMPYLOBACTER LABORATORY SURVEY

In 2005 ESR conducted a survey of Campylobacter isolation techniques conducted by New Zealand laboratories. The results of this survey are being prepared for publication and cover the 2004 calendar year. Portions of the Campylobacter survey collected data that are comparable to that collected during the 2006 AGI Laboratory survey for the 2005 calendar year. This section compares these data.

4.1 Campylobacter Survey Response and Laboratory Type

The Campylobacter survey was sent to 64 laboratories, and 51 responses were received for analysis (80% response). Prior to the AGI survey, all potential participating laboratories were contacted, including those involved in the Campylobacter survey, and it was found that a number had closed. Additionally, a number of laboratories were not included in the AGI survey as they were a collection point for specimens and did not do the testing on-site or tested veterinary or industrial specimens only. This is the source of the disparity in the number of laboratories involved in each survey (47 in the AGI survey, 64 in the Campylobacter survey).

Most (51%) of the laboratories responding to the Campylobacter survey described themselves as a 'hospital-based laboratory'. A further 37% described themselves as community laboratories, and 9% as hospital and community laboratories. The remainder selected either 'commercial reference' (2%) or 'government' (2%). When compared to the AGI survey, the responding laboratories made up similar proportions (46% Hospital only, 34% Community only, 14% Hospital and Community, 6% Public Health; Section 3.2.1).

4.2 Testing Faecal Specimens for Campylobacter

4.2.1 Number of faecal specimens tested

For the Campylobacter survey, 41 laboratories provided data on the number of faecal specimens tested for Campylobacter during 2004. These data are compared with the AGI results in Table 16.

Table 16: Comparison of the number of faecal specimens tested for Campylobacter from the two surveys

Data	Campylobacter survey (2004 data)	AGI survey (2005 data)
No. responses	41	26
Total specimens tested	177,319	108,016
Mean	4,325	4,154
Median	1,677	1,267
Max	58,520	48,143
Min	14	47

4.2.2 Criteria for testing

The AGI survey found that almost all laboratories routinely test faecal specimens for Campylobacter. Similarly in the Campylobacter survey, of the 43 laboratories that selected testing criteria, 88% indicated that they routinely tested faecal specimens for *Campylobacter* spp.. A

further 9% tested by request from a physician, and one laboratory noted that they only test for Campylobacter as part of a food poisoning investigation.

4.2.3 Discarding specimens

In the AGI survey, participants were asked for the reasons that specimens might be discarded without testing. In the Campylobacter survey, participants were specifically asked about rejection criteria relating to specimen age and patient hospitalisation. These data can be compared.

In the Campylobacter survey participants were asked if there was a length of time from stool specimen collection to receipt in the laboratory after which the specimens would not be tested. Of the 35 laboratories responding to this question, 51% did not have any such criteria. For the remaining 17 laboratories, 12 quoted the time for rejection as 24 hours, two as 72 hours, one as 48 hours, one as 18 hours and the last did not provide a rejection time. The AGI survey, where participants were asked to list their own criteria, also demonstrated that the age of the specimen was an important reason for a specimen not being tested, and the time before rejection varied between 24 and 72 hours.

Participants of the AGI survey also specified that specimens would be rejected if they were from a patient that was hospitalised for more than three days, though testing for *C. difficile* toxin might occur instead of culturing. In the Campylobacter survey, 17 laboratories indicated that they had rejection criteria based on the length of hospitalisation, with 13 laboratories rejecting after 3 days hospitalisation and the remainder after 4 days.

4.2.4 Testing methods

The Campylobacter survey requested very detailed information on the testing methods used in the laboratories.

Selective media

In the Campylobacter survey, 40 laboratories provided information on the type of selective media they use for directly plating faecal specimens to isolate Campylobacter. The media most commonly listed was CAT, which was used by 40% of the laboratories, followed by CCDA or mCCDA, listed by 30% of laboratories. Skirrows was used by 10% of respondents from the Campylobacter survey (one mentioning that it was modified). With the exception of non-specific responses such as ‘Campylobacter isolation agar’, the other selective media listed in the Campylobacter survey were only used by 1-2 laboratories and included charcoal-based selective medium (CSM), Campy BAP (Blaser or Campy agar with five antimicrobials) and Exeter agar.

Enrichment

In the Campylobacter survey, no laboratories reported doing any enrichment for Campylobacter, which is equivalent to the findings of the AGI survey.

Incubation temperature and conditions

When asked about the temperature at which Campylobacter plates are incubated, 90% of the 42 respondents to this question incubated their plates at 42°C. Three laboratories (7%) used 37°C and one laboratory incubated at 40°C. From the AGI survey, 90% of laboratories also incubated at 42°C (the other temperatures were more variable).

4.3 Supplementing AGI Survey with Data from the Campylobacter Survey

Although the Campylobacter survey concerned the preceding calendar year to the AGI survey, it is possible to extract some information that can fill gaps in the AGI survey. Of the 11 laboratories that did not return a questionnaire for the AGI survey, seven responded to the Campylobacter survey. These seven laboratories reported receiving 18,781 faecal specimens for Campylobacter testing in 2004. If it is assumed that Campylobacter testing was routine for all faecal specimens, and that the number of faecal specimens received for testing by a laboratory per year is relatively constant, then this could be added to the total number of faecal specimens provided during 2005, giving a total of approximately 203,000 specimens per year.

Of the 35 laboratories who responded to the AGI survey, nine did not provide an estimate for the number of specimens tested for Campylobacter (Q15.1). Eight of these laboratories provided this number for 2004 in the Campylobacter survey. These laboratories tested 41,125 faecal specimens for Campylobacter.

These 41,125 specimens partially fill the gap between the total number of faecal specimens (184,252 reported by 34/35 laboratories, Table 2) and the number of specimens tested for Campylobacter (108, 016 reported by 26/35 laboratories, Table 7).

5 DISCUSSION

This report is principally intended to summarise results from the laboratory survey. Analysis of the data is limited. This is because all three AGI study elements need to be considered together to assess the influences on reporting of the illness. It is intended that an overall draft report, integrating the results from all three studies, will be provided at the end of June 2007, to be finalised by the end of August 2007.

5.1 Response Rate

The response rate for this survey was lower than expected, given that each laboratory had been contacted directly and agreed to participate. From anecdotal comments by non-responding laboratories, it appeared that they may have been inhibited from responding by the extremely competitive commercial environment which pervaded the laboratory sector during the survey period.

However, the different laboratory types are well represented, and good geographical coverage suggest that the results are representative of the sector as a whole.

5.2 Stool Specimen Numbers, and Numbers of Individuals from whom Samples were Received

The number of samples, when augmented with data from the Campylobacter survey, suggest that in excess of 200,000 samples were submitted in 2005. Using the mean value of 5,419 from 34 laboratories (Table 2) and scaling up to the full complement of 46 laboratories suggests that the number of samples may actually be approximately 250,000. The number of actual samples may be even higher, given that a number of community laboratories did not complete the survey, and such laboratories may handle large numbers of samples (Table 2).

The number of laboratories reporting the number of individuals from whom samples were received was much lower than those reporting sample numbers. The average sample:individual ratio was 1.4:1, and was highest for community laboratories (1.9:1).

As might be expected, approximately 77% the samples derive from people presenting to primary health care providers, while the majority of the remainder are from patients in hospitals.

5.3 Testing Decision Making

The majority of samples are accompanied by a test request form, but the testing requested is often in general terms (e.g. “culture”). Figure 4 indicates that laboratories are not informed of the individuals symptoms for all samples, but if they are supplied, the information is more likely to influence the testing performed. Responses to testing criteria for individual types of pathogens suggests that the patient’s age, travel history, and condition of the specimen were more influential than the symptoms themselves.

Discarding of specimens appears to be infrequent, and occurs mostly because the sample is unsuitable for testing, either physically or administratively.

5.4 Testing Performed

It appears that, unless instructed otherwise, laboratories will test faecal specimens for the bacterial pathogens Campylobacter, Salmonella, Yersinia and Shigella, and the parasites Giardia and Cryptosporidium. Tests for *E. coli* O157, Vibrio, rotavirus and *C. difficile* toxin might also be performed depending on the clinical symptoms or age of the patient, and Aeromonas can be detected as part of routine culture for the other bacterial pathogens. The pathogenicity of Aeromonas remains to be determined.

5.4.1 Bacteria

Testing for the bacterial pathogens Campylobacter, Salmonella, Yersinia and Shigella is conducted at least five times more frequently than other bacteria. Samples are ten times less likely to be tested for STEC or *E. coli* O157, and *Vibrio* spp., while testing for *Listeria* spp., *Bacillus* spp., *Clostridia* spp., and *Staphylococcus aureus* is performed on very few samples. When the survey of GPs is completed, a comparison between their expectations of testing and actual tests can be conducted.

The infrequency of testing faecal specimens for *Bacillus* spp., *Clostridia* spp., and *Staphylococcus aureus* is unlikely to be a cause of under-reporting for at least two reasons:

- Faecal shedding of *Bacillus* spp. and *Clostridia* spp. occurs in a proportion of healthy people; it is only elevated numbers of bacteria which may indicate causation of AGI; and,
- Many incidents of AGI caused by these organisms result from the ingestion of preformed toxin in foods (*Bacillus* emetic toxin, *Staphylococcus aureus* toxins), and the organism may not appear in faeces.

Listeria monocytogenes may cause a non-invasive febrile illness and the infrequency of testing for Listeria may mask the occurrence of this illness. The low frequency of testing for STEC and *Vibrio* spp. may also cause some cases of AGI caused by these organisms to be undetected, although the occurrence of bloody diarrhoea will prompt testing for STEC (see below).

In terms of criteria for bacterial testing, the majority of testing is conducted as routine screening procedures, with some tests performed when requested. A number of laboratories reported that some tests were not available; principally *Enterobacter sakazakii*, *Bacillus* spp, *Clostridia* spp. and *Staphylococcus aureus*. Laboratory decision making was most frequently reported for STEC (decision based on bloody diarrhoea, clinical symptoms, or age of patient) and *Vibrio* spp. (decision based on liquid/fluid sample, clinical details, travel or seafood consumption).

The methods reported for detection of bacteria indicate that there is considerable variation across laboratories in terms of enrichment, agar, and incubation temperatures. None of the methods reported would be ineffective, but the experience of the laboratory worker would be a factor in the recognition of some bacteria.

Approximately 1000 more samples were reported as being tested for Salmonella compared to the other routine bacterial pathogens (Table 7). This might be for a variety of reasons, including samples tested as clearance for occupational reasons, repeat testing for chronic infections or long term carriage.

5.4.2 Viral pathogens

Only a small proportion of faecal samples are tested for enteric viruses. Approximately 10% of samples are tested for rotavirus, about 1.5% for adenovirus, and less than 0.5% for norovirus. Decision making for testing for enteric viruses was evenly split between request or laboratory criteria. As expected, the laboratory criteria for rotavirus testing was largely based on the age of the patient. Testing of samples from young children was most common, although the maximum age of the children varied considerably.

Testing of faecal samples for norovirus most commonly occurs in an outbreak situation, and only a few samples are likely to be tested as indicative. This will therefore miss the endemic burden of norovirus infection.

Methods for detection of enteric viruses appeared to be suitable.

5.4.3 Parasites and protozoa

Approximately a quarter of all faecal samples were tested for ova, while approximately a third were tested for Giardia and Cryptosporidium. Tests for a wide range of other parasites are conducted, but only on a small number of samples (perhaps 1% of the total).

Testing for parasites and protozoa was reported as routine in only a few laboratories; most perform this type of testing only on request, while some laboratories report making their own decisions. Based on reported sample numbers however, parasite testing is requested frequently. The laboratory criteria used were mostly related to clinical details, while recent overseas travel was important for conducting ova testing, and the sample being from a child was important for Giardia and Cryptosporidium testing.

There was considerable variation in the testing methods reported for parasites and protozoa. Laboratory worker experience would be a key factor in successful detection of the pathogens, particularly for microscopy methods.

5.4.4 Toxins

Testing of faecal samples for toxins is rare. Testing is most often performed for *C. difficile* toxin, with perhaps 7% of samples tested. This test is more likely to be performed on samples from hospitalised patients, because *C. difficile* is a common cause of colitis and/or diarrhoea following antibiotic intake in a hospital or care facility.

5.5 Specimen Storage and Further Testing

A high proportion of laboratories (31/35) store samples, with the most common time period being a week. Refrigerated storage was most common.

5.6 Non Detection of Pathogens

It is expected that for a large number of samples, no pathogen will be detected. Apparently laboratory systems are not well set up to provide this type of information, but data from 17 of the laboratories indicate that no pathogens are detected in two thirds or more of samples.

One reason for absence of pathogens is that the sample is from a person who is seeking a "non-detection" result to enable resumption of normal activities. The number of these samples is difficult to estimate. Although one laboratory did report a high proportion of such samples, opinion from other laboratory workers is that the number of such "clearance" samples is low.

Other reasons for non-detection of pathogens include:

- Pathogen present but not tested for (most likely);
- Pathogen not being shed in faeces (information reviewed in Appendix 2 on the period of shedding in relation to symptoms suggests that this is unlikely for most bacterial pathogens and norovirus, but could be relevant for toxin producing bacteria)
- Unsuitability of the sample for testing (although most laboratories report that samples are examined and discarded if unsuitable).

Although increasing the range of tests conducted on samples is likely to reduce the proportion of samples in which no pathogen is detected, it is worth noting that despite a comprehensive testing regime for bacteria, viruses, and parasites on all samples obtained in the United Kingdom Infectious Intestinal Disease Study, in approximately half the samples no pathogen could be detected (Wheeler *et al.*, 1999).

5.7 Further Testing and Sample Referral

Most of the laboratories who responded indicated that no further testing was performed on samples from which no pathogen was identified, and no samples are referred to the ESR Public Health Laboratories. From the remaining laboratories, the number of samples subjected to further testing was very low, and most likely to be from children.

There are likely to be economic barriers to such further testing, which may be more expensive, and not covered by routine funding arrangements.

In contrast, referral of samples to the virology laboratories at ESR was reported by 24/33 of the laboratories that responded.

The referral of isolates to the ESR Enteric Reference Laboratory was routine for nearly all laboratories in the case of isolation of Salmonella, Shigella and STEC, and for approximately half the laboratories when *Vibrio* spp. or Yersinia isolates were obtained. It would be useful if all isolates of these two pathogens were routinely sent to the Enteric Reference Laboratory. The low numbers of laboratories referring isolates of *Listeria* spp. or *Enterobacter sakazakii* possibly reflects the low numbers of laboratories conducting testing for these bacteria.

5.8 Reporting

Healthlink (a computer network) or print reporting formats were most common for sending results to GPs and Public Health Units. Direct contact (by telephone) between the laboratory and test requestor would most often occur to clarify testing requirements, or report results of public health significance (e.g. the isolation of pathogens with serious adverse outcomes).

5.9 Comparison with Overseas Surveys

5.9.1 National Studies on Acute Gastrointestinal Illness (NSAGI): 2001 Canadian Laboratory Study (Flint, 2002)

This survey was administered to 470 microbiology laboratories across Canada; 408 (87%) responded. The study revealed a number of variations in criteria for testing stool specimens. A small proportion (35) of samples were rejected due to the absence of transport media, the stool being fully formed, or the container being damaged or contaminated. Routine testing for *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* and *Yersinia* was conducted by all, or almost all laboratories. Other pathogens, such as *Plesiomonas* and *Vibrio* were routinely tested by fewer laboratories (54% and 38% respectively).

Overall 459982 stool specimens were tested for enteric bacterial pathogens (excluding *C. difficile*), 177696 for *C. difficile*, 392023 for enteric parasites, and 14051 for enteric viruses. The population of Canada in 2001 was 31.0 million. On average, 5.0%, 7.6%, 15.3% and 18.9% of stool specimens tested for bacteria, parasites, *C. difficile*, and viruses were positive. The study author assumed that the total number of cases submitting stool samples was equal to the total number of stools submitted for bacterial testing, and thus the overall proportion of cases for which a pathogen was identified was 29.4%.

5.9.2 FoodNet sites in the USA (Voetsch et al.*, 2004)*

A survey of clinical laboratories in the US FoodNet sites, from 1995-2000, indicated that testing for Salmonella, Shigella and Campylobacter was routine for all samples, but only 57% tested for *E. coli* O157:H7, 50% for *Yersinia enterocolitica* and 50% for *Vibrio* species. The proportion of samples that yielded these bacterial pathogens was Campylobacter (1.3% of specimens) Salmonella (0.9% of specimens), Shigella (0.4% of specimens) and *E. coli* O157:H7 (0.3% of specimens).

5.10 Estimated Numbers of Faecal Samples and Testing Results for New Zealand

To construct an estimated national overview for New Zealand, it is necessary to extrapolate from the information obtained by the survey. The calculations are based on the following:

Faecal samples submitted:

A total of 184,252 samples were reported by 34 laboratories. Based on publicly available information, the remaining laboratories were assigned to a category. Missing were results from:

6 Hospital laboratories
5 Community laboratories
1 Hospital and community laboratories

Using the results in Table 2, this suggests total samples for New Zealand of 218,970 (using median values) or 256,471 (using mean values). The differences between the mean and median values in Table 2 indicate that the distributions for sample numbers are skewed. The largest contributor to sample numbers are the community laboratories. Since there were several major community laboratories that did not respond to the survey, the value derived from the means in Table 2 is considered more reliable.

A total of 256,471 samples represents a rate of 0.06 samples per person per year for a New Zealand population of 4,098,900 (based on Statistics New Zealand population estimates for 2005).

Faecal samples discarded:

The information on Section 3.4.4 indicates that few laboratories were able to provide quantitative data for this question. Of the laboratories that did respond, the answers were 0% (7 laboratories), <1% (4 laboratories), 5.5%, 7.1%, <13.6%. The mean value from these responses is approximately 2%.

Faecal samples tested:

Based on the above information, this suggests that 251,341 samples were tested.

Pathogen found in community faecal samples:

The estimate in Section 3.9.2.1 is that pathogens were found in approximately 20% of samples from cases in the community. This represents up to 50,000 samples (0.01 samples per person per year).

In comparison there were approximately 18,000 notified cases of infection with bacterial and parasitic pathogens in 2005. This excludes cases caused by enteric viruses or *C. difficile*, which are major causes of acute gastroenteritis, but are not notifiable.

6 CONCLUSION

A previous report (Sarfati et al., 1997) indicates that patients (over 5 years) with AGI symptoms presenting to general practitioners (GPs) in New Zealand are requested to provide stool samples in:

- less than 25% of cases by 42% of general practitioners;
- 25 – 50% of cases by 31% of GPs; and,
- over 50% of cases by 23% of GPs.

This appears to be similar to other countries, where a range of 14 – 27% of those seeking medical care with an acute diarrhoeal illness are asked to submit a stool sample (Scallan et al., 2006). Using the estimated 256,471 stool samples submitted in 2005, of which an estimated 77.1% derived from primary healthcare providers (Table 4), there may be up to 791,000 GP consultations by people in New Zealand with AGI symptoms. This estimate should be treated with caution however; this survey did not ask laboratories to differentiate between stool samples submitted by patients with AGI and those submitted for other reasons.

The same New Zealand survey indicated that routine stool culture (Salmonella, Shigella, and Campylobacter) was the most commonly requested test, followed by rotavirus, Yersinia, Giardia and Cryptosporidia. Norovirus testing was rarely requested. This situation appears to be unchanged, although Yersinia now appears to be included in the routine culture testing. Despite advances in methods for detecting norovirus, testing is still requested for only a small proportion of samples, most often in outbreak situations. This is likely to contribute to the health burden of AGI caused by endemic infection with norovirus being under-recognised.

There is considerable variation in the methods used for all the pathogens examined in this study. It is likely that if pathogens are present, then they will be present in high numbers and sensitivity (and enrichment, for bacterial pathogens) should be less important. Therefore variation in methods may not be a significant factor in detection.

7 REFERENCES

De Wit MA, Koopmans MP, Kortbeek LM, van Leeuwen NJ, Bartelds AI, van Duynhoven YT. (2001) Gastroenteritis in sentinel general practices, the Netherlands. Emerging Infectious Diseases; 7: 82-91.

Flint J. (2002) Report of the 2001 Canadian Laboratory Study. National Studies on Acute Gastrointestinal Illness. Division of Enteric, Foodborne and Waterborne Diseases. PPHB, CIPDC, Health Canada.

Sarfati D, Bates MN, Garret N, Baker MG. (1997) Acute gastroenteritis diagnostic practices of New Zealand general practitioners. New Zealand Medical Journal; 110; 354-356.

Scallan E, Jones TF, Cronquist A, Thomas S, Frenzen P, Hoefer D, Medus C, Angulo FJ, and the FoodNet Working Group. (2006) Factors associated with seeking medical care and submitting a stool sample in estimating the burden of foodborne illness. Foodborne Pathogens and Disease; 3: 432-438.

Voetsch AC, Angulo FJ, Rabasky-Her T, Shallow S, Cassidy M, Thomas SM, Swanson E, Zansky SM, Hawkins MA, Jones TF, Shillam PJ, Van Gilder TJ, Wells JG, Griffin PM. (2004) Laboratory practices for stool-specimen culture for bacterial pathogens, including *Escherichia coli* O157:H7, in the FoodNet sites 1995-2000. Clinical Infectious Diseases; 38 (Suppl 3): S190-197.

Wheeler JG, Sethi D, Cowden JM, Wall PG, Rodrigues LC, Tompkins DS, Hudson MJ, Roderick PJ. (1999) Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. British Medical Journal; 318: 1046-1050.

APPENDIX 1: LABORATORY SURVEY FORM

National Acute Gastrointestinal Illness Study

LABORATORY SURVEY

Conducted by: The Institute of Environmental Science and Research (ESR)

For the: New Zealand Food Safety Authority (NZFSA)

Endorsed by: The Ministry of Health

30 June 2006

Note: The information provided by this survey will be held confidentially by ESR and the NZFSA. Any results will be reported anonymously and aggregated.

Thank you for taking the time to complete this survey, it is most appreciated.

Please complete the survey by **19 July 2006** and return in the stamped addressed envelope to:

Rob Lake
Christchurch Science Centre
ESR
PO Box 29 181
Christchurch

If you have any queries relating to this survey please contact Rob Lake:

By email: Rob.Lake@esr.cri.nz
By phone: (03) 351 0048

1

Section A: Laboratory and Contact Details

Laboratory ID code:

Name of laboratory:

Address/City/Postcode:

Person completing questionnaire:

Position:

Phone number:

Date questionnaire completed:

Which group, if any, of laboratories is your laboratory affiliated with?

Section B: General Information

1. Which of the following best describes your laboratory?

- ☐ Hospital-based
- ☐ Community diagnostic
- ☐ Public health

2. Approximately, what geographical region(s) is served by your laboratory?

Section C: Stool Specimen Collection and Transport

3. How many stool samples did your laboratory receive during the period 1 January to 31 December 2005?

4. How many individuals did these samples come from?

Section D: Stool Specimen Sources

5. Approximately, what proportion of your stool specimen testing is requested by the following sources?

Hospital health care professionals		%
Primary health care providers		%
Occupational health care providers		%
Public Health Services		%
Direct requests from the public		%

Section E: Decision Making Regarding Testing Samples for Enteric Pathogens

6. Do samples arrive with the required testing specified on a standardised laboratory form?

☐ Always (100%)	☐ Usually (80-99%)	☐ Sometimes (21-79%)	☐ Rarely (1-20%)	☐ Never (0%)

7. We are interested in the specifics of how doctors request faecal testing. Are you able to provide us with copies of the last 10 faecal specimen submission forms you received (anonymised)?

- ☐ Yes, anonymised copies enclosed with this survey
- ☐ Sorry, not able to provide

8. Is the laboratory made aware of a patient's symptoms?

☐ Always (100%) ☐ Usually (80-99%) ☐ Sometimes (21-79%) ☐ Rarely (1-20%) ☐ Never (0%)

9. Does knowledge of a patient's clinical details influence your testing regimen?

☐ Always (100%) ☐ Usually (80-99%) ☐ Sometimes (21-79%) ☐ Rarely (1-20%) ☐ Never (0%)

10. If you receive a sample where the only request is for "faecal (or enteric) pathogens", what tests would your laboratory perform on the sample?

11. How many samples were discarded in 2005 before any testing was performed?

12. For what sorts of reasons are samples discarded without being tested?

Section F: Testing of Stool Samples for Enteric Bacteria

Please complete the following table, indicating for each bacteria the number of samples tested in 2005, and the criteria and testing methods your laboratory uses for testing for that particular bacteria. Note: listed testing methods may not be applicable for some bacteria.

Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
13. *Salmonella* spp.	13.1 #:	13.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	13.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)
14. *Shigella* spp.	14.1 #:	14.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	14.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)

Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
15. Campylobacter spp.	15.1 #:	15.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	15.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)
16. Yersinia spp.	16.1 #:	16.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	16.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)
17. Listeria spp.	17.1 #:	17.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	17.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)
18. STEC including *E.Coli* O157:H7 NB. Isolates NOT toxin	18.1 #:	18.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	18.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)

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Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
19 Aeromonas spp.	19.1 #:	19.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	19.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)
20. *Vibrio* spp.	20.1 #:	20.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	20.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)
21. Plesiomonas spp.	21.1 #:	21.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	21.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)
22. Enterobacter sakazakii	22.1 #:	22.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	22.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)

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Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
23. Bacillus spp. NB. Isolates NOT toxin	23.1 #:	23.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism NB. If you do test for *Bacillus spp.* please list the particular species that you test for here:	23.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)
24. Clostridium spp. NB. Isolates NOT toxin	24.1 #:	24.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism NB. If you do test for *Clostridium spp.* please list the particular species that you test for here:	24.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)
25. Staphylococcus aureus NB. Isolates NOT toxin	25.1 #:	25.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	25.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)

Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
26. Other enteric bacteria (please specify)	26.1 #:	26.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable	26.3 ☐ Selective media (Please specify) ☐ Enrichment ☐ Incubation at specific temps (Please specify) ☐ Other method(s) (Please specify)

Section G: Testing of Stool Samples for Enteric Viruses

Please complete the following table, indicating for each virus the number of samples tested in 2005, and the criteria and testing methods your laboratory uses for testing for that particular virus. Note: listed testing methods may not be applicable for some viruses.

Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
27. Rotavirus	27.1 #:	27.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	27.3 ☐ Latex agglutination ☐ EIA ☐ PCR ☐ Culture ☐ Other method(s) (Please specify)
28. Norovirus	28.1 #:	28.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	28.3 ☐ Latex agglutination ☐ EIA ☐ PCR ☐ Culture ☐ Other method(s) (Please specify)

Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
29. Hepatitis A NB. In stool NOT serology	29.1 #:	29.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	29.3 ☐ Latex agglutination ☐ EIA ☐ PCR ☐ Culture ☐ Other method(s) (Please specify)
30. Other enteric viruses (please specify)	30.1 #:	30.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable	30.3 ☐ Latex agglutination ☐ EIA ☐ PCR ☐ Culture ☐ Other method(s) (Please specify)

Section H: Testing of Stool Samples for Parasites and Protozoa

Please complete the following table, indicating for each organism the number of samples tested in 2005, and the criteria and testing methods your laboratory uses for testing for that particular organism. Note: listed testing methods may not be applicable for some organisms.

Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
31. Ova	31.1 #:	31.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	31.3 ☐ Direct microscopy ☐ Antigen testing ☐ Immunofluorescence ☐ EIA ☐ PVA/SAF ☐ Other method(s) (Please specify)

Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
32. Giardia	32.1 #:	32.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	32.3 ☐ Direct microscopy ☐ Antigen testing ☐ Immunofluorescence ☐ EIA ☐ PVA/SAF ☐ Other method(s) (Please specify)
33. Cryptosporidium	33.1 #:	33.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this organism	33.3 ☐ Direct microscopy ☐ Antigen testing ☐ Immunofluorescence ☐ EIA ☐ PVA/SAF ☐ Other method(s) (Please specify)
34. Other parasites/ protozoa (please specify)	34.1 #:	34.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable	34.3 ☐ Direct microscopy ☐ Antigen testing ☐ Immunofluorescence ☐ EIA ☐ PVA/SAF ☐ Other method(s) (Please specify)

Section I: Testing of Stool Samples for Toxins

Please complete the following table, indicating for each toxin the number of samples tested in 2005 and the criteria your laboratory uses for testing for that particular toxin.

Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
35. Staphylococcus aureus	35.1 #:	35.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this toxin	35.3 Details not required
36. Bacillus cereus	36.1 #:	36.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this toxin	36.3 Details not required
Clostridium perfringens	37.1 #:	37.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this toxin	37.3 Details not required

Organism	# Samples Tested in 2005	Criteria for Testing	Testing Methods Used (Tick all that apply)
38. Clostridium difificile	38.1 #:	38.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this toxin	38.3 Details not required
39. STEC including *E.coli* O157:H7	39.1 #:	39.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable, do not test for this toxin	39.3 If you test for shiga-toxin, please specify which kit you use ☐ Not applicable, do not test for shiga toxin
40. Other toxin. (please specify)	40.1 #:	40.2 ☐ Test routinely as part of enteric screen ☐ Only test if specifically requested by referring doctor ☐ Laboratory has own criteria for testing (please specify) ☐ Not applicable	40.3 If you test for other toxins please specify testing method

Section J: Sample Storage and Further Testing

41. Are samples stored pending further testing requests?

☐ Yes ☐ No

If yes, how are they stored?

If yes, for how long are they stored?

42. In 2005, for how many samples that you tested (i.e. excluding discarded samples) was no pathogen identified?

43. If no pathogen is identified from a sample, is further testing sometimes performed?

☐ Yes ☐ No

If yes, under what circumstances would further testing occur?

If yes, approximately, how many samples had further testing performed in 2005 after initial testing was all negative?

44. Does your laboratory refer samples to either of ESR's Public Health laboratories (PHL) in Auckland and Christchurch for additional testing?

☐ Yes ☐ No ☐ Not applicable (PHLs only)

If yes, what are the criteria?

45. Does your laboratory refer faecal samples to any of ESR's virology laboratories in Wellington for additional testing?

☐ Yes ☐ No

If yes, please specify for which viruses this is done?

46. Which bacterial isolates do you send to ESR's Enteric Reference Laboratory (ERL) in Wellington for further typing?

Isolate	Send to ERL	
Salmonella spp.	☐ Yes	☐ No
Shigella spp.	☐ Yes	☐ No
Yersinia spp.	☐ Yes	☐ No
STEC	☐ Yes	☐ No
Vibrio spp.	☐ Yes	☐ No
Enterobacter sakazakii	☐ Yes	☐ No
Other isolate(s) (please specify)		

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Section K: Reporting of Results

47. How are results usually sent to (you may tick more than one box):

a. GPs?

- ☐ Healthlink (HL7)
- ☐ Printed report
- ☐ Email
- ☐ Fax
- ☐ Phone
- ☐ Other (specify)

b. Public Health Unit /Medical Officer of Health (if relevant)?

- ☐ Healthlink (HL7)
- ☐ Printed report
- ☐ Email
- ☐ Fax
- ☐ Phone
- ☐ Other (specify)

c. ESR (if relevant)?

- ☐ Healthlink (HL7)
- ☐ Printed report
- ☐ Email
- ☐ Fax
- ☐ Phone
- ☐ Other (specify)

48. Are there any circumstances where someone from your laboratory would contact a faecal sample test requestor directly by phone?

☐ Yes ☐ No

a. If yes, please specify

49. Are there any circumstances where someone from your laboratory would contact a Medical Officer of Health or Public Health Unit directly by phone regarding faecal sample test results?

☐ Yes ☐ No

a. If yes, please specify

Thank you for completing this survey. Please return in the post-paid envelope supplied.

APPENDIX 2: FAECAL SHEDDING IN RELATION TO AGI

Literature information about period of shedding in relation to illness:

- Of 105 people associated with an outbreak of *Salmonella* Typhimurium in Japan, stool specimens were monitored until 2 clear specimens were obtained from 33 symptomatic and 18 asymptomatic people. *S.* Typhimurium was recovered from most asymptomatic people at 12 days post-exposure, but not after. This was the similar for the 10 symptomatic people who were not hospitalised, however three of these individuals still had positive stools at 25 days post-exposure. For most of the symptomatic individuals who were hospitalised (many of which were given antibiotics), positive stools were still being collected beyond 30 days post-exposure, and one patient did not return a negative stool specimen until after 112 days post-exposure. Murase, T., Yamada, M., Muto, T., Matsushima, A. & Yami, S. (2000) Fecal excretion of *Salmonella enterica* serovar Typhimurium following a food-borne outbreak. *Journal of Clinical Microbiology* 38(9): 3495-3497.
- After the onset of diarrhoea caused by *E. coli* O157, stool samples were collected from 53 children until three specimens were negative within 9 days. The median duration of shedding was 13 days, ranging from 2 to 62 days in patients with diarrhoea or hemorrhagic colitis. The median duration was 21 days in patients with HUS, range 5 to 124 days. In 36 (68%) of the patients, only the first stool was positive (median collection of 1st sample after diarrhoea was 3 days for non-HUS and 9 days for HUS), and in 7 (13%) of patients stools were still positive after 32 days (all asymptomatic by this stage). Karch, H., Rüssmann, H., Schmidt, H., Schwarzkopf, A. & Heesemann, J. (1995) Long-term shedding and clonal turnover of enterohemorrhagic *Escherichia coli* O157 in diarrheal diseases. *Journal of Clinical Microbiology* 33(6): 1602-1605.
- Stools were taken daily from 20 volunteers administered varied doses of non-toxigenic *Listeria monocytogenes* over 14 days in hospital, then 6 additional weeks as outpatients. After administration, *L. monocytogenes* was detected in stools from 15/20 individuals within 1 day. All but one subject shed bacteria for 4 days or less. Angelakopoulos, H., Loock, K., Sisul, D.M., Jensen, E.R., Miller, J.F. & Hohmann, E.L. (2002) Safety and shedding of an attenuated strain of *Listeria monocytogenes* with deletion of *acta*/*plc*B in adult volunteers: A dose escalation study of oral inoculation. *Infection and Immunity* 70(7): 3592-3601.
- A study of diarrhoeal episodes in Egyptian infants found that *Campylobacter* was still excreted for a month after the diarrhoeal episode. The organism is shed for an average of 2-3 weeks after cessation of diarrheal symptoms in cases from the developed world compared with durations as short as 1 week in children from developing areas. Rao, M.R., Naficy, A.B., Savarino, S.J., Abu-Elyazeed, R., Wierzba, T.F., Peruski, L.F., Abdel-Messih, I., Frenck, R & Clemens, J.D. (2001) Pathogenicity and convalescent excretion of *Campylobacter* in rural Egyptian children. *American Journal of Epidemiology* 154(2): 166-173.
- Norovirus shedding: Of 5 elderly people infected with norovirus, the median excretion time was 8.6 days, range 2-15 days (Goller, J.L., Dimitriadia, A., Tan, A., Kelly, H. & Marshall, J.A. (2004) Long-term features of norovirus gastroenteritis in the elderly. *The Journal of Hospital Infection* 58(4): 286-291). After challenge with norovirus, shedding was common after infection and was present up to 2 weeks (Okhuysen, P.C., Jiang, X., Ye. L., Johnson, P.C. & Estes, M.K. (1995) Viral shedding and fecal IgA response after Norwalk virus infection. *The Journal of Infectious Diseases* 171(3): 566-569). For volunteers challenged with norovirus, the peak of viral shedding was 25-72 h, virus first appeared in stool at 15 h, specimens collected 7 days after inoculation remained positive (Graham, D.Y., Jiang, X., Tanaka, T., Opekun, A.R., Madore, H.P. & Estes, M.K. (1994) Norwalk virus infection of

volunteers: New insights based on improved assays. *The Journal of Infectious Diseases* 170(1): 34-43.

- Rotavirus shedding: Of infants admitted to hospital, shedding of rotavirus was over 1-5 days (Gaggero, A., Avendaño, L.F., Fernández, J. & Spencer, E. (1992) Nosocomial transmission of rotavirus from patients admitted with diarrhea. *Journal of Clinical Microbiology* 30(12): 3294-3297).
- Yersinia shedding: Where onset of symptoms known, *Y. enterocolitica* was still detected mean 50.4 days after (median 40; range 17-116). Mean interval between first and last positive stool was 37.6 days (range 10-93). Ostroff, S.M., Kapperud, G., Lassen, J., Aasen, S. & Tauxe, R.V. (1992) Clinical features of sporadic *Yersinia enterocolitica* infections in Norway. *The Journal of Infectious Diseases* 166: 812-817.