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Audit of stool testing performed in a microbiology laboratory: will using fixed predefined clinical criteria for stool testing of *Giardia* and *Cryptosporidium* lead to missed cases?

Sir,

Giardia lamblia (intestinalis or duodenalis) and *Cryptosporidium* are protozoan parasites which frequently cause diarrhoeal illness. Both have been associated with frequent outbreaks in community and institutionalised settings.[1,2] Several modalities for detection of these parasites are available, ranging from microscopy, antigen assays, enzyme immunoassays (EIA) and multiplex polymerase chain reaction (PCR). Faecal microscopy is time-consuming, operator-dependent and insensitive for detection of these parasites in low burden setting. Faecal testing using other modalities like antigen assay, EIA and multiplex PCR are able to detect these parasites and are incorporated depending on the laboratory size and workflow.

Most microbiology laboratories across Australia have individualised stool testing protocols with some routinely testing for *Giardia/Cryptosporidium* on all specimens with community-onset of diarrhoeal illness while others testing only when there is clinical information/history available. The usual history that prompts such testing is that of persistent or chronic diarrhoea (symptoms $\geq$2 weeks), recent overseas travel and diarrhoea in an immunosuppressed patient, patient from a refugee health clinic or at the request of the local public health unit (these are 'predefined clinical criteria' for our microbiology laboratory).[3] Variations in testing exist as some laboratories perform a broad range multiplex PCR upfront on stool specimens, some perform EIA or antigen-based tests and others faecal parasite multiplex PCR. For the laboratories that do not perform multiplex PCR on all stool specimens, testing for *Giardia/Cryptosporidium* is done at the clinician's request and/or if the predefined clinical criteria are included on the pathology request form. The above testing strategies were found to be widespread after consultation with the representatives from the microbiology laboratories within NSW Health and a few other larger laboratories across Australia.

Electronic ordering is now standard in most Australian hospitals. Figure 1 shows the sequential appearance of the 'pop-up' boxes during the electronic ordering of stool tests. While the 'clinical history' field is crucial, this field (middle box in Fig. 1) can be bypassed with a single character computer entry and other useful information like immunosuppression, recent overseas travel, and onset are not mandatory fields. For laboratories performing *Giardia/Cryptosporidium* testing based on predefined clinical history/criteria, the absence of this information creates difficulty for the laboratory to determine appropriate tests.

We had the opportunity to audit the stool testing in our microbiology laboratory as the faecal testing for *Giardia* and *Cryptosporidium* species was performed on all specimens [except cases of hospital-onset of diarrhoea (diarrhoea $\geq$72 hours after admission)] over a 4-year period (2014–2017). Clinical details of the cases with *Giardia* and *Cryptosporidium* were obtained from electronic medical records (eMR). Ethics approval was obtained as a quality improvement audit. The microbiology laboratory uses Giardia/Cryptosporidium Quik Chek assay (Abbott Diagnostics, USA) for detection. The manufacturer reports the sensitivity and specificity as 97.6% and 100%, respectively. In literature, this test performs better than conventional stool microscopy; however, multiplex PCR has shown even better results with specificity.[4,5] The cost of consumables/test for this assay is AU$10.70.

Over a 4-year period (2014–2017), 7162 faecal specimens were examined in our laboratory. Of these, 35 were positive (non-duplicate) for *Giardia lamblia* and 19 for *Cryptosporidium* (total *n*=54). This gave a 'pick-up' rate for this test of 0.75%. Clinical records were available for 52 of 54 patients (two patients were excluded from analysis). Based on the pathology request forms and electronic orderable only 30% (16/52) of these infections would have been detected. These 16 patients had a history of chronic diarrhoea or had a 'travel history'. Therefore, we reviewed clinical records of all available patients with positive results (*n*=52) to determine further history, treatment given, and outcomes including resolution of symptoms and/or recurrence of an episode. Characteristics and findings of these patients are summarised in Table 1.

Based on information gathered from the eMR audit, parasite testing should have been performed on 25/52 (48%) patients if appropriate history and information were available to the laboratory at the time of performing the test. These additional cases (*n*=9) were patients with chronic diarrhoea (duration >2 weeks), history of travel and immunosuppression, of which four patients represented with recurrent symptoms and were eventually treated. While it is difficult to gauge the clinical impact of performing such testing (and missing the diagnosis) on a retrospective audit, overall, we found that six patients (12%, including the four patients mentioned above) represented back to the emergency department with ongoing symptoms after an initial presentation to the hospital. Definitive therapy with metronidazole and nitazoxanide was prescribed in 12/52 (23%) patients after results of these tests were available to the clinicians, which would not have happened if the *Giardia/Cryptosporidium* testing was carried out. Data on resolution could be obtained on only 32 patients due to the retrospective nature of the audit. All 32/52 (61%) patients showed complete resolution of symptoms. Empirical therapy with metronidazole was only given in 13/52 (25%) patients after presentation with their symptoms as the initial presenting symptoms were thought to be either viral or non-infective.

We also retrospectively extracted information concerning stool consistency from the laboratory database to determine if this could provide a useful laboratory marker to direct testing. The retrospective review of stool consistency showed that 7.4% (530/7162) of all stools and 11% (6/54) of positive stools were categorised as 'formed'. This indicates that if formed stools are used as an exclusion criterion one would still have to test 92.6% of all stools and 11% of positives would be missed. Currie *et al.* and Lindo *et al.* also found that

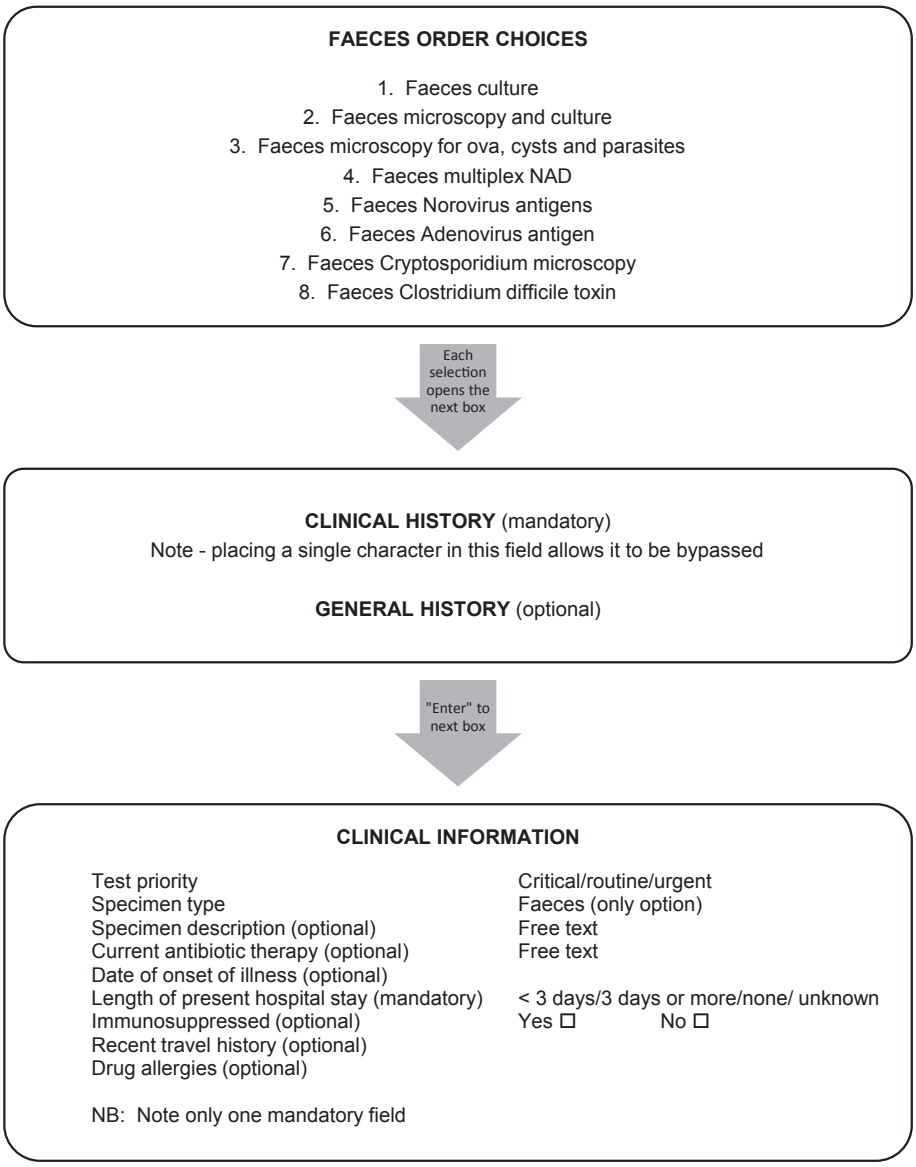

Fig. 1 Sequential appearance of 'pop up' boxes during electronic ordering of faeces testing.

patients who have infections with *Giardia* do not necessarily have unformed stools; they present with other symptoms like abdominal pain and could be missed if formed stools are used as an exclusion criterion.[6,7]

Two United Kingdom national audits also found that testing based on clinical history would lead to underreporting of several cases of *Giardia* and *Cryptosporidium* which in turn could potentially lead to long term sequelae of untreated infection and outbreaks.[8,9] Also, in one of these studies, the acquisition of *Giardia* was deemed to be locally acquired from food and water sources. It is known that livestock, petting zoos and other water sources can be contaminated with cysts of *Giardia*.[3,10] While treatment may not need to be administered for every case of *Giardia* and *Cryptosporidium* infection, patients with ongoing symptoms and those who are immunosuppressed would be appropriate for treatment. Treatment with suitable antimicrobials is known to shorten the duration of symptoms, shedding of the cysts and reduce morbidity.[11–14] Although the clinical impact of opportunistic detection of protozoan parasites seems to be small in our study (12 patients were treated after positive results), there are consequences of chronic infection leading to increased morbidity as mentioned above and public health implications.[15] We think that a simple cost-benefit analysis would not be representative of the issue of laboratory workload and cost of consumables weighed against morbidity associated with such illness. While more staff time would be required to process all stool specimens versus selected specimens, we feel that laboratories would still wish to process all faeces specimens if they were missing more than 50% of these cases based on predefined screening criteria. The implications of missing positive cases [morbidity, lost productivity, sick days, investigations (i.e., endoscopies) performed, overall health and community aspects of such illness] are impossible to quantitate in this retrospective study and in real life.

With the availability of several commercial faeces multiplex PCR assays, which include targets for bacteria (including *Clostridium difficile*), viruses and parasites including *Giardia* and *Cryptosporidium*, a single assay or a standalone faecal parasite PCR assay would be adequate to detect all such infections. Incorporation of such molecular testing for all faeces specimens (not >72 hours) into the

Table 1 Characteristics of patients with faecal parasitic infections

Variable	Giardia lamblia (*n*=34)	Cryptosporidium spp (*n*=18)
Mean age, years ± SD	46.3 ± 26.5	24.2 ± 21
Length of stay, mean days	6.7	2.2
Overseas travel	2	4
Immunosuppression	2	2
Chronic/persistent diarrhoea[a]	11	0
Faeces microscopy (wet)	11	1
Another bacterial pathogen isolated on stool concurrently	2	1
Presentation with non-diarrhoeal symptoms[b]	11	2
Empirical therapy metronidazole	8	5
Definitive therapy metronidazole	10	0
Definitive therapy nitazoxanide	0	3
Symptomatic treatment only[c]	16	14
Patients who represented to ED following discharge due to ongoing symptoms	3	3
Resolution of symptoms[d]	21	11
Resolution attributed to antibiotics (impact)	9	3

[a] Chronic/persistent was mentioned on the patient history in eMR, pathology orderable or pathology request form.
[b] Fever, bloating and abdominal pain.
[c] Intravenous fluid, antispasmodic and antimotility drugs.
[d] Details from electronic record.

workflow of the microbiology laboratory would improve efficiency (high throughput and quicker turnaround time) and avoid the missed clinical cases seen with predefined criteria-based testing. The clinicians could then elect to treat those patients who do not resolve their symptoms quickly or those who are immunosuppressed. We acknowledge that in certain cases (returned travellers with diarrhoea, refugees/migrants), faecal multiplex PCR or EIA for *Giardia/Cryptosporidium* alone may not be adequate. These patients would benefit from extended faeces testing by microscopy with permanent stains and other tests for detection of other parasitic and helminth causes of chronic diarrhoea.

In summary, when using predefined clinical criteria to test for *Giardia* and *Cryptosporidium*, 70% of these cases would have been missed based on the laboratory request form. Even in cases where clinical information was actively sought from the eMR, about 52% of cases were missed. Not testing formed stool specimens would result in missed cases without too much reduction in the laboratory workload of faecal testing. We believe the only way of improving detection of *Giardia* and *Cryptosporidium* is to perform testing on all cases of community-onset diarrhoea. Although antigen detection/EIA may be useful in some small regional laboratories, broad panel multiplex PCR or faecal parasite PCR should be the preferred test in other laboratories.

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Lymphocyte involvement in nivolumab-induced autoimmune myositis

Sir,
Nivolumab is an IgG4 monoclonal antibody to PD-1 that is currently approved for treatment of patients with advanced non-small cell lung cancer (NSCLC) who experience progression of disease after platinum-based chemotherapy. Common immune mediated adverse events include rash, diarrhoea/colitis, hepatitis, pneumonitis and hypothyroidism. We report a case of biopsy proven immune-mediated myositis after a single dose of nivolumab.

A 61-year-old male with a background history of metastatic NSCLC (*KRAS*, *EGFR*, *ALK* and *ROS1* wild type) presented to a rural hospital with a 5 day history of upper and lower limb weakness, and difficulty swallowing after being