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Stakeholder submission on behalf of Histopath Diagnostic Specialists – Options for the Regulation of Faecal Microbiota Transplantation (FMT) materials

Section: FMT testing

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Histopath is a NATA accredited diagnostic practice, and is regarded as a centre of excellence for microbial, viral, parasite and biochemical testing of faecal specimens. Our practice has been regularly screening potential FMT donors for more than 10 years, has performed screening tests on greater than 1000 donor samples during the past 18 months and has a close working relationship with clinicians performing FMT.

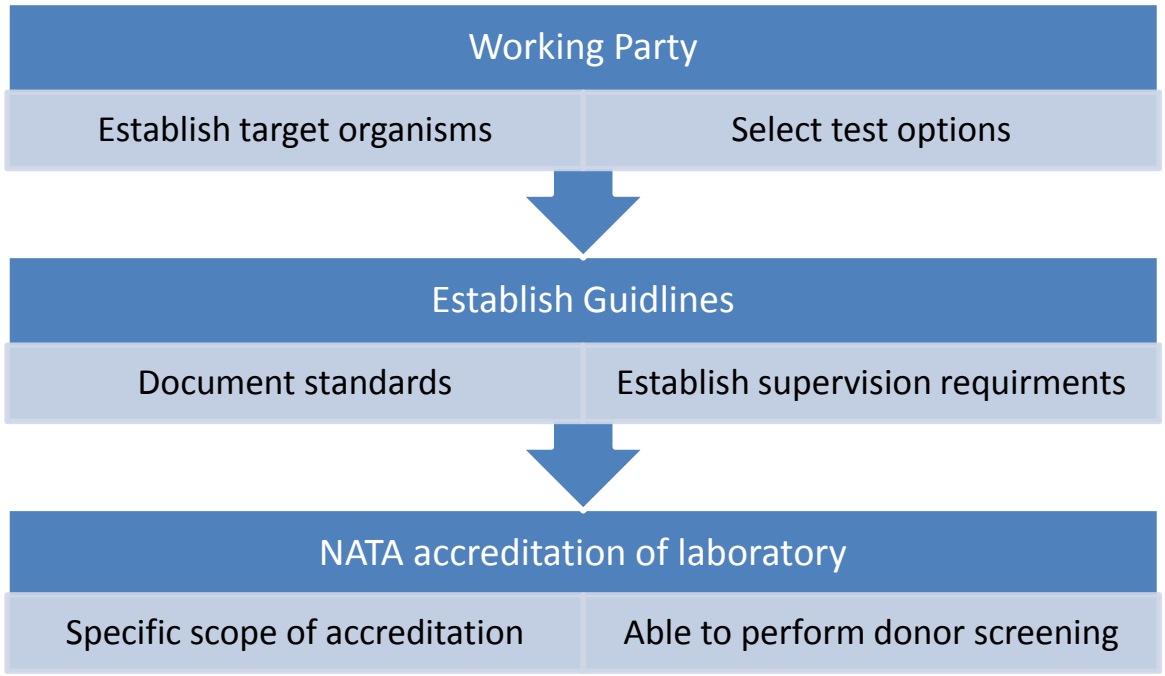
FMT is regarded as a safe treatment. Transmission of infection is very rare, even in immunocompromised patients. Asymptomatic carriage states of viruses and pathogenic bacteria are rare. The most potential infective agents detected on donor stool screening are parasites, particularly ***D. Fragilis and B. hominis***. In our practice, during the past 12 months, one (0.1% of all donor samples) donor sample tested positive for Adenovirus, which subsequently cleared on later testing and 85 (approximately 10% of all donor samples) samples tested positive for at least one parasite. No donor samples tested positive for pathogenic bacteria. Preliminary data from the recent introduction of MDRO screening suggests positivity in up to 10% of donors.

General comments regarding the Regulation of Faecal Microbiota Transplantation (FMT) materials

The current approach suggested in the draft document appears to be directed at validating specific assays / tests for screening purposes. It is likely that most of the assays / tests involved cannot be validated as screening tests in a timely or cost effective manner due to the extremely low incidence of asymptomatic carriage rates for the target organisms in healthy patients. Even if the process is

achievable, this process would offer no tangible benefit for patients or clinicians, and in fact may result in greater cost, which in turn may limit access to treatment. There needs to be a focus on the screening process, rather than on individual tests. At this stage, there is no accreditation of donor stool screening. There is currently a shortage of experienced parasitologists in Australia and expertise in this area of testing is deficient in many laboratories. Hence there is a drive away from traditional reference tests involving microbial culture and microscopy by parasitologists, towards immunoassays and molecular techniques that are for diagnostic purposes only. While requiring less expertise, these newer techniques have significant limitations in the diversity of organisms that can be targeted and will miss some potential pathogens if used for screening. For screening purposes therefore, these tests should be regarded as supplementary to traditional reference tests, and not alternatives.

Variations in the quality of screening for donor stools, arises primarily from a lack of standardisation of the overall process. Screening of donor stools should be regarded as a specialised area, requiring supervision by experienced microbiologists and parasitologists and adherence to appropriate standards. If regulation is to be applied, it needs to assess and regulate donor screening as a specialised diagnostic scope, rather than regulate individual assays.



The most appropriate approach is therefore to design screening guidelines and protocols for each target, once organism targets are established. A working group should be involved in establishing standards for selection of methods and protocols. Limitations of testing need to be quantified and disclosed to clinicians and patients. Diagnostic facilities must demonstrate that testing is supervised by appropriately trained microbiologists and parasitologists. Standards and procedures need to be documented, and assessed by an appropriate body. The most appropriate body in Australia is NATA, and donor screening of stool specimens should be accredited as a specific scope of practice.

Responses to specific questions related to screening of donor stool samples.

Q15. *Are the proposed timeframes for the initial collection of blood and stool samples for testing and the frequency of repeat collections and testing for donors, appropriate? If not, then please provide justification for alternative requirements.*

Time frames are appropriate, however there needs to be clarification of the number of stool samples required for testing.

Section 9 'Requirements in relation to donor blood and stool testing'. Page 37. The wording of (8) should be modified. "A stool sample" which implies a single specimen, should be changed to "Stool samples" as three (3) stool specimens are required for infectious disease screening in order to provide appropriate sensitivity. This particularly applies to parasite examination due to irregular shedding of organisms. For this test, sensitivity is only 60% with single specimen, but increases to >95% with three specimens. In our practice collection of 3 stools on separate occasions over 2-3 days is recommended, which is in line with the recommendations of CDC (6).

Q16. *If changes to current FMT practice are required to comply with these requirements, what is the expected cost impact? Is a 12 month transition period sufficient to update current practice? Please provide enough information to allow us to understand any impact*.

There is no Health insurance or Medicare rebate for FMT in Australia. Cost of the procedure is high and availability is low. Any added cost related to assay validation would further restrict patient access.

Q17. *A definitive solution is not provided here as to the appropriate validation of test methods used on stool samples for FMT products, but is the proposed pathway for continued engagement with the sector appropriate?*

From a practical point of view, validation of tests currently used for donor stool screening will yield little success and be of no benefit to patients. Instead, the solution should involve establishing standards and protocols for stool screening. This would provide clarity to the sector and form a framework to base accreditation. This would provide a greater opportunity to provide patients with options, greater understanding and improved outcomes

Q18. *Is the requirement for testing to occur under contracted arrangements currently occurring? If not, then are there any problems or anticipated costs associated with establishing this arrangement?*

In general, contractual arrangements for diagnostic tests may impair free market drivers of quality. It is however agreed that a close working relationship must exist between the diagnostic service and

clinicians. Risks and limitation in screening must be disclosed and documented so that patients can be fully informed. It is essential that all donor stool tests are supervised by a microbiologist experienced in stool testing and that all parasite microscopic examination be performed by an expert parasitologist (7).

Q19. *Is archiving of blood and stool samples currently being undertaken? If not, are there any problems or anticipated costs associated with establishing this arrangement?*

There is no routine archiving of stool donor samples.

No current testing methods are validated for examination of frozen stool specimens. The current RCPA guidelines stipulate that "stool specimens should never be frozen". Some organisms are affected by freezing and the testing of frozen stool samples may prove to be unreliable.

Q21. *Strongyloides stercoralis testing*.

As an alternative to serology, an experienced parasitologist performing microscopy for parasites will include examination for Strongyloides larvae. Providing three specimens are received, sensitivity will be similar at 70-80% (6). A supplementary PCR test for stool is also available.

Q24. *Is there a need to test for Norovirus and Rotavirus?*

This is a difficult question to answer. There are reports in the literature of Norovirus following FMT, despite negative donor screening. It is very difficult to determine if the infection has been transmitted from the donor or has been acquired from a community source. It is likely, however that given the choice, most patients would elect to reduce the risk as much as possible if donor screening is available.

Q25. *Entamoeba histolytica*

This parasite is uncommon, but not rare in Australia, with a carriage rate of 2% from immigrants. In addition, other species of *Entamoeba* may be pathogenic, such as *E. moshkovskii*. Many carriers will be asymptomatic, however transmission of the parasite to a donor may result in active disease with colitis / liver abscess and screening of donors is therefore justified. *E. histolytica* is very transmissible, although the infectious dose is unknown, in theory, just one viable cyst is sufficient to establish infection (9). The reference for screening is microscopy performed by a qualified and experienced parasitologist. No ELISA or molecular tests (PCR) tests are validated for screening, and while useful to increase sensitivity for *E. histolytica*, they will not detect *E. moshkovskii*. In our practice microscopy is used as the primary screening test but is routinely supplemented by the addition of PCR. A Sydney hospital study, found over a 4 year period, 3% of samples had cysts and/ or trophozoites microscopically resembling *E. histolytica/ dispar/ moshkovskii* (2). The differentiation of *E. histolytica* from the rest of the complex is possible by molecular techniques, available at our institute and found to be of great value. Given that *E. histolytica* is endemic in Sydney, it is important to screen for it in these samples. Considering that it has been reported that *E.moshkovskii* can be

pathogenic, use of molecular testing alone will not detect this parasite. Its presence can only be detected by microscopy and an expert parasitologist (7).

Q28. *Should any of the stool tests listed above under 'other criteria', or any others which are relevant, be included in the draft TGO?*

As per the table on page 53 (copied here with), test agents (highlighted in yellow) should be amalgamated as they all test for the same microorganisms. When all 3 are combined as one test, it is observed that it is a test recommended in all other consensus statements and should be included in the requirements for FMT testing. We recommend that parasite testing is performed by microscopy (class 1 IVD test) which is also the gold standard and will identify ALL intestinal parasites include *E. moshkovskii*. *Isospora and Microsporidia* should be deleted as *Isospora* is included in parasite examination and *Microsporidia* has it's section as a microorganism.

Microorganisms and other test agents	Australia	EU	US	UK	International
Yersinia spp.	No	Yes	No	No	Yes
Methicillin-resistant staphylococcus aureus	No	Yes	No	No	No
Gram-negative multidrug-resistant bacteria	No	Yes	No	No	No
Protozoa (including Blastocystis hominis) and helminths and parasites	No	Yes	No	No	Yes
Faecal occult blood testing	No	Yes	No	No	No
Vibrio cholera	No	Yes	No	No	Yes
Listeria monocytogenes	No	Yes	No	No	No
Isospora and Microsporidia	No	Yes	No	No	Yes
Calprotectin	No	Yes	No	No	No
Adenovirus	No	No	Yes	No	Yes
Ovum and parasite microscopic examination	No	No	Yes	Yes	No
Microsporidia microscopic examination	No	No	Yes	Yes	No
Isospora and Cyclospora microscopic examination	No	No	Yes	Yes	No

Of particular importance to exclude are the following:

D. fragilis, B hominis – asymptomatic carrier states are common in healthy patients, and transfer to a recipient is possible. There is evidence that these organisms may result in significant morbidity, requiring treatment that may be, prolonged, difficult and expensive. The organisms therefore satisfy the requirements for inclusion in the TGO. In our experience, these two organisms are by far the most common pathogen detected in donor stools.

Isospora* and *Cyclospora – Each can exist in asymptomatic carriers but can cause acute debilitating illness when active. Both are easily detected on using stool microscopy by an expert parasitologist.

Note: Stool microscopy will also identify the presence of white cells and red cells in the stool which can be indicators of inflammation or bleeding, for example from inflammatory bowel disease or cancer.

Comment about *H.pylori*. Although controversial, there is no definitive evidence that viable *H. pylori* exists in the stools of asymptomatic people. In addition, screening options for donor stools are very limited. No Class 4 validated tests are available for screening donor stools for *H. pylori*. Our practice does provide a molecular test, which can be used if the organism is included in the TGO, however this test is not readily available at other laboratories. An antigen test is available, but has limited sensitivity.

Histopath recommendations for TGO:

Microorganism	Reference Test*	Additional tests***		
Clostridiodes difficile	Culture	PCR		
Salmonella spp.	Culture	PCR		
Shigella spp.	Culture	PCR		
Campylobacter spp.	Culture	PCR		
Giardia duodenalis	Microscopy	PCR		
Cryptosporidium spp.	Microscopy	PCR		
Entamoeba histolytica	Microscopy	PCR		
Helminths and intestinal parasites	Microscopy			
Norovirus	Nil**	EIA / PCR		
Rotavirus	Nil**	EIA / PCR		
Enterovirus	Nil**	EIA / PCR		
MDROs including MRSA, VRE, ESBL and CRE	Culture	PCR (optional supplement)		
Helicobacter pylori	Nil**	PCR (limited availability)		
B. hominis, D. fragilis, Isospora, Cyclospora	Microscopy	PCR		

*These tests should be exempt from the Class 4 requirement as they use microbial culture and microscopy performed by an experienced, qualified parasitologist.

**No practical or cost effective reference tests are available as routine screening tests

***These tests are currently all validated for diagnostic use only, and should be regarded as supplementary

References:

1. Hiatt RA, et al. Am J Trop Med Hyg., How many stool examinations are necessary to detect pathogenic intestinal protozoa?, The American Journal of Tropical Medicine and Hygiene, Volume 53, Issue 1, 1 Jul 1995, p. 36 – 39

2. Sebastiaan J van Hal, Damien J Stark, Rashmi Fotedar, Debbie Marriott, John T Ellis and Jock L Harkness, Amoebiasis: current status in Australia, Med J Aust 2007, 186 (8), p. 412-416

3. Woodworth *et al*, Laboratory testing of donors and stool samples for fecal microbiota transplantation for recurrent Clostridium difficile infection. Journal of Clinical Microbiology, April 2017, Vol 55 (4), p. 1002-1010

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8. R. Fotedar, D. Stark, N. Beebe, D. Marriott, J. Ellis, and J. Harkness, PCR Detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* in Stool Samples from Sydney, Australia, Journal of Clinical Microbiology, 2007 March, p. 1035–1037

9. Irving E. Salit, Krishna Khairnar, Kevin Gough and Dylan R. Pillai A Possible Cluster of Sexually Transmitted *Entamoeba histolytica:* Genetic Analysis of a Highly Virulent Strain, Clinical Infectious Diseases 2009; 49:346–53