FAECAL BACTERIA AND PARASITES 12-WELL
REF 25041

PANEL OF ASSAYS

CONSISTING OF THE IVD COMPONENTS:
STEP 1 TUBES FOR FAECAL BACTERIA AND PARASITES 12-WELL REF 25041S
STEP 2 PLATES FOR FAECAL BACTERIA AND PARASITES 12-WELL REF 25041P

FOR THE HIGHPLEX

INSTRUCTIONS FOR USE

IVD ARTG IVD CE

These Instructions for Use (IFU) must be read in conjunction with the Highplex IFU.

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1. WARNINGS AND LIMITATIONS

- **IMPORTANT**: Do not use if product or packaging is compromised in any way.
- **IMPORTANT**: Do not use Step 1 or Step 2 panel components with different catalogue and/or version numbers.
- **IMPORTANT**: Do not use expired products.
- Always handle and dispose of specimens potentially containing human pathogens according to relevant safety procedures.
- This panel of assays must only be used with the Highplex.
- Good laboratory practice is essential for the intended performance of this product. For further safety information, please consult the relevant Safety Data Sheets (SDS). **Note**: No AusDiagnostics reagents contain hazardous substances, as listed in Regulation (EC) No 1272/2008[1] and according to the Globally Harmonised System (GHS) classification. AusDiagnostics SDS's can be accessed online at http://www.ausdiagnostics.com/regulatory.html
- This panel of assays is designed to measure specific nucleic acid sequences. Therefore, a negative result does not exclude the possibility that an unusual sequence variant is present. The results obtained with this product should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. A negative result cannot be relied upon for a definitive diagnosis. Failure or delay in treatment of an infected patient may lead to death, with immunocompromised patients being at highest risk.

2. FURTHER INSTRUCTIONS REQUIRED

The Instructions for Use (IFU) for Faecal Bacteria and Parasites 12-well includes product-specific information not provided in other IFUs. The document ID of the IFU for this product is provided on the outer box label next to the IFU symbol and in the footer of this document. The IFU for this product can be downloaded from the URL printed on the outer box label. Additionally, a paper copy is available upon request by contacting customer service via phone, email, or mail (see **Section 12. Technical Enquiries**).

These Instructions for Use must be read in conjunction with the IFU for:

- Highplex system (REF 91501)
- Low DNA Reagent Cassette (REF 40231)

and if applicable:

- Synthetic Positive Controls for Faecal Panels (REF 91031)

3. NAME AND INTENDED USE

Faecal Bacteria and Parasites 12-well is intended for use by suitably trained personnel in qualified laboratories using the Highplex (REF 9150).

These tests utilise a multiplex-tandem polymerase chain reaction (MT-PCR)[2] for the enrichment of targets and then amplification of targeted DNA and/or RNA. For the full description of the principle of the method, see the Highplex IFU, **Section 5. Principle of Method.**

Faecal Bacteria and Parasites 12-well is intended as a semi-automated test for the identification of pathogens in nucleic acid extracts from appropriate specimen types. For specimen and sample types that may be used, please see **Section 6. Specimen Requirements**.

The pathogens targeted in this panel are listed and described in **Section 4. Summary and Explanation of the Test.**

4. SUMMARY AND EXPLANATION OF THE TEST

FAECAL BACTERIA AND PARASITES 12-WELL REF 25041 VER 02

ARTG Identifier: 236377

Assay	Target
Salmonella + Shigella	Including Most *Salmonella enterica* and *S. bongori, Shigella spp*. (includes all 4 serotypes and some EIEC strains; excludes EHEC).
Campylobacter	*C. jejuni, C. coli, and C. doyeli*; excludes *C. hominis*.
E.coli O157	*Escherichia coli* O157:H7.
C.diff toxin B	*Clostridium difficile* toxin B containing strains
C.diff toxin A	*Clostridium difficile* toxin A containing strains
Yersinia	*Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (includes all isolates)
Shiga toxin	Includes Shiga toxin and Shiga-like toxin 1 and 2 (from *Shigella dysenteriae* and STEC). Will not detect Stx2f variant.*
Cryptosporidium	*Cryptosporidium spp*. (includes *C. parvum, C. hominis, C. wrairi, C. meleagridis*; excludes *C. tyzzeri, C. baileyi, C. felis*).
Giardia	*Giardia lamblia* (includes assemblages A through F).
E.histolytica	*Entamoeba histolytica* (excludes other *Entamoeba* e.g. *E. dispar* and *E. moshkovskii*).
Sample Adequacy	Human reference gene for sample adequacy control.
SPIKE	Artificial sequence for assay control.

*The assay is designed to detect ST2a, b, c, and d only.

These tests utilise a multiplex-tandem polymerase chain reaction (MT-PCR)[2] for the amplification of targeted DNA or RNA. For the full description of the principle of the method, see the Highplex IFU, **Section 5. Summary and Explanation of the Test.**

The Faecal Bacteria and Parasites 12-well panel is intended to detect the following bacteria and parasites that have been associated with gastrointestinal infection:

4.1 TARGET DESCRIPTIONS: BACTERIA AND TOXINS

Salmonella

Salmonella in humans can cause gastroenteritis, bacteraemia and blood infections. Non-typhoidal Salmonella (including *S. enteritidis* and *S. typhimurium*) are the dominant causes of Salmonella infections, while typhoidal *S. typhi* and *S. paratyphi* are the most common causes of enteric fever. Low numbers of Salmonella may also be present in the faeces of healthy asymptomatic carriers. The Salmonella assay is designed to detect both enteric fever and non-typhoidal serotypes of Salmonella. The assay detects most common pathogenic subspecies of *Salmonella enterica*. The assay does not detect *S. arizonae*.

Shigella spp.

Shigella spp. infections are caused by any of the four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. The bacterium is highly contagious due to its low infectious dosage (as few as 10 organisms) and the fact that it is transmitted by direct or indirect contact with faecal matter[3]. Differentiating *Shigella spp*. from *E. coli* is a major problem faced by laboratories as the two are closely related[4]. The Shigella assay

is designed to detect all four Shigella serotypes and some enteroinvasive *E. coli* (EIEC). It excludes enterohaemorrhagic *E. coli* (EHEC).

Campylobacter spp.

Campylobacter spp. are the most common bacterial cause of food-borne diseases in developed countries, a consequence of widespread colonisation in poultry farming and the consumption of undercooked poultry products[5, 6]. 90% of human *Campylobacter spp*. infections are due to *C. jejuni* and *C. coli*[6]. This assay is designed to detect *C. jejuni*, *C.coli*, and *C. doyeli*. It excludes *C. hominis*.

Escherichia coli O157

Escherichia coli O157 is the most commonly identified Shiga toxin-producing *E. coli* (STEC)[7]. Infection is most commonly associated with consuming contaminated food particularly meat, causing severe, acute hemorrhagic diarrhoea. The *E. coli* O157 assay is designed to detect the most commonly identified STEC *E. coli* strain O157:H7.

Clostridium difficile

Clostridium difficile infection commonly occurs after normal gut microbiota is disrupted by antibiotic treatment[8]. *C. difficile* pathogenicity is mainly attributed to the production of the toxin B (TcdB) and Toxin A (tcdA).

A third toxin, binary toxin has also been observed in some pathogenic *C. difficile* strains[9]. The *C. difficile* assay is designed to detect all strains containing the toxin B gene. The tcdA assay is designed to detect all strains containing the toxin A gene. A third toxin, binary toxin has also been observed in some pathogenic *C. difficile* strains. The *C. difficile* assay is designed to detect all strains containing the toxin B gene

Yersinia

Yersinia species infections in humans can cause gastroenterititis which may lead to sepsis, particularly in immunocompromised persons.[3, 10]. Humans can become infected with Yersinia enterocolitica and Yersinia pseudotuberculosis due to contaminated food, milk, water or pig products. *Y. pseudotuberculosis* is rarer then infection caused by other Yersinia pathogens such as *Y. pestis* and *Y. enterocolitica*[10].

Shiga toxins

Shiga toxins are genetically and structurally related cytotoxins produced by *Shigella dysenterieae* and STEC, including E. coli strains O157:H7 and O145[11]. STECs may also be referred to as verocytotoxin-producing *E. coli* (VTEC) or EHEC. Shiga toxins are divided into Shiga toxin 1 and 2, and their genetic variants (Shiga toxin-like), which can cause outbreaks of bloody diarrhoea after ingestion of contaminated food[12]. This assay is designed to detect Shiga toxins and Shiga-like toxins 1 and 2 (Stx2a-e only. Excludes Stx2f.).

4.2 TARGET DESCRIPTIONS: PARASITES

Cryptosporidium spp.

Cryptosporidium spp. can cause profuse watery diarrhoea, with *C.hominis* and *C.parvum* the most frequently isolated species in human cases[13]. Infection has a seasonal variation, and is a particular problem with the immunocompromised[14]. The Cryptosporidium assay is designed to detect *Cryptosporidium spp*. including *C. parvum* and *C. hominis*.

Giardia lamblia

Giardia lamblia (synonymous with *G. intestinalis* and *G. duodenalis*) is the most common intestinal parasite in humans, causing diarrhoea, bloating and malabsorption. Giardia is divided into genetic assemblages, where assemblages A and B are commonly reported to infect humans[15]. The Giardia assay is designed to detect *G. lamblia* including assemblages A to F.

Entamoeba histolytica

Entamoeba histolytica is globally considered a leading parasitic cause of human mortality, with ~100,000 associated deaths each year[16]. It can cause amoebic dysentery and invasive extra-intestinal amoebiasis. *E. histolytica* is morphologically identical to the other non-pathogenic members of the Entamoeba genus that are found in the human intestinal lumen (e.g. *E. dispar* and *E. moshkovskii*)[14, 16]. The *E. histolytica* assay is designed to detect *E. histolytica* and excludes other Entamoeba such as *E. dispar* and *E. moshkovskii*.

5. PANEL COMPONENTS: MATERIALS AND STORAGE

Panel Name	Panel Components	REF	GTIN
Faecal Bacteria and Parasites 12-well	Step 1 Tubes for Faecal Bacteria and Parasites 12-well	25041S	9343044003189
	Step 2 Plates for Faecal Bacteria and Parasites 12-well	25041P	9343044003172
To be used with:	Low DNA Reagent Cassette	40231DNA	9343044003080

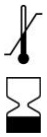
Note: No AusDiagnostics reagents contain hazardous substances, as listed in Regulation (EC) No 1272/2008[1] and according to the Globally Harmonised System (GHS) Classification.

5.1 STEP 1 TUBES

Step 1 Tubes for Faecal Bacteria and Parasites 12-well contain tubes for 96 samples.

Materials	Label	Description	Function	Qty.
Step 1 Tubes	**STEP 1 TUBES**	1 x 96-slot plastic frame containing 12 x 8-well tube strips with dried oligonucleotides.	Receptacle for the Step 1 reaction	96

STORAGE AND HANDLING INSTRUCTIONS



The Tandemplex® Step 1 tubes are delivered with a 12-month shelf life providing the product is refrigerated at 2° to 8°C on arrival, per the label instructions. Product is delivered at room temperature.

If the product is being partially used, the product may be removed from the refrigerator to remove the required Step 1 tubes but remaining product must be returned to the refrigerator within 30 minutes.

The Tandemplex® Step 1 tubes may also be stored on arrival at ambient temperature (14° - 29° C) but the shelf life is reduced to 6 months from the date of manufacture. In the case of ambient temperature storage, the provided labels must be applied to the product to indicate the ambient storage temperature and reduced shelf life.

IMPORTANT: Once the product is stored at room temperature for beyond 30 minutes, it cannot be stored back in the refrigerator and the room temperature expiry storage expiry will apply which is 6 months from the date of manufacture. Variations to the stipulated storage of the product may lead to false or inconclusive results.

5.2 STEP 2 PLATES

The Step 2 Plates box for Faecal Bacteria and Parasites 12-well contains materials for 288 samples.

Materials	Label	Description	Function	Qty.
Step 2 Plates box	**STEP 2 PLATES**	Outer box	Outer packaging	1
Step 2 Plate	**STEP 2 PLATE**	Sealed bag containing 384-well plate with dried oligonucleotides	Receptacle for the Step 2 PCR reaction	12
Dilution plate	(D)	Empty, 96-well plate	Houses the dilution for Step 2	6

STORAGE AND HANDLING INSTRUCTIONS

The Step 2 Plates box must be stored between 14°C - 29°C.

IMPORTANT: Ensure desiccant pouches are intact before removing product from sealed bag; do not use product if desiccant is compromised or missing

Expiration of product is 6 months from manufacture and expiration date is provided on the label.

5.3 MATERIALS REQUIRED BUT NOT PROVIDED

Required reagents and equipment that are not provided by AusDiagnostics are:

- Personal protective equipment (PPE)
- Bleach with 0.4% available chlorine (4 mL required per run)
- Nuclease-free and adjustable pipettes

5.4 ADDITIONAL CONSUMABLES REQUIRED BUT NOT PROVIDED

- Robot tips, ZTF-100-R-S, Carton (50 racks of 96) (REF 93250)
- Robot tips, ZTF-100-R-S, Carton (10 racks of 96) (REF 93210)
- Tip disposal bags (100) for Highplex (REF 91502)
- Bleach tubes (60) for Highplex (REF 91503)
- Pack of 6 Dilution Plates, Foil Sealed (REF 90020)
- Sealing films for MT-PCR assay plates (REF 90201)
- Low DNA Reagent Cassette (REF 40231)

Please contact AusDiagnostics to purchase these consumables (**see Section 12. Technical Enquiries**).

6. SPECIMEN REQUIREMENTS

6.1 SPECIMEN TYPES AND VOLUME

A nucleic acid extract that is suitable for PCR should be used with this product. Acceptable specimen types include nucleic acid extracts of faecal samples and culture.

PCR inhibitors may be present in nucleic acid extracts from faecal samples and their level is dependent on the method used (see **Section 6.2**). Nucleic acid extracts should be free of particulate matter.

Note: please store your nucleic acid extract in tubes free from PCR inhibitors and nucleases

AusDiagnostics has validated the use of Roche S.T.A.R. buffer and Qiagen Buffer ASL – stool lysis buffer (according to the manufacturers 'Instructions for Use) for the preparation of fresh, non-preserved faecal specimens to produce nucleic acid extracts suitable for use with AusDiagnostics Faecal panels:

Take approximately 100-200 µL of fresh faecal sample into 1 mL of the buffer, vortex thoroughly and freeze. - Thaw the sample and heat to 95°C for 10 minutes.

Centrifuge for 3 - 5 minutes at 1000 x g.

For the MT-Prep extractor use 200 µL of the supernatant for nucleic acid extraction. Alternatively, use the volume specified by the extractor instructions

IMPORTANT: long term storage of un-extracted faecal samples in buffer (even when stored frozen) may result in degradation of RNA. When working with RNA samples, standard precautions to minimise RNA degradation should be used.

IMPORTANT: Always handle and dispose of specimens potentially containing human pathogens according to relevant safety procedures.

Volume of sample to be added to Step 1 tube must be 10 µL

6.2 SUITABLE NUCLEIC ACID EXTRACTION METHODS

Manual extraction and pipetting of nucleic acid extracts into Step 1 Tubes is best performed in a biological safety cabinet or PCR setup area.

The following nucleic acid extraction methods have been validated by AusDiagnostics customers and have been deemed suitable to produce nucleic acid extracts compatible with the Faecal Bacteria and Parasites 12-well panel of assays. For further details on the extraction protocols used, please contact AusDiagnostics (**see Section 11. Technical Enquiries**).

Extraction System	Protocol Name	Type
AusDiagnostics MT-Prep Extractor	MT-Prep Virus/ Pathogen Extraction kit B	Automated
bioMerieux NucliSENS easyMAG	QiaAmp One-For-All Nucleic Acid kit	Automated
Qiagen QIAsymphony	DSP/Virus/Pathogen Mini Kit	Automated
Qiagen EZ1	EZ1 DSP Virus Kit	Automated
Qiagen Universal Biorobot system	QiaAmp One-For-All Nucleic Acid Kit	Automated
Roche High Pure series	High Pure Viral Nucleic Acid Kit	Manual
Roche MagNA Pure 24	Total NA Isolation Kit	Automated
STRATEC Molecular InviGenius	InviMag® Universal Kit/IG	Automated
Abbot m2000sp	mSample Preparation System DNA	Automated

7. FURTHER MT ASSAY SET UP OPTIONS

This section is intended to be read in conjunction with the Highplex IFU, **Section 7.3 Run the Processor**.

Additional MT Assay Setup software options are as follows:

Sampling: The table below explains the options provided for robotic sampling:

Sampling options	Applicable situation
Manual pipetting into tube strip	Nucleic acid extracts have been manually transferred to the Step 1 Tubes prior to starting the run (therefore no robotic sampling required).
Robot sampling from 5 mL tubes	Specimens eluted into 5mL Tubes (2 mL buffer) (REF 90210) are loaded on the Autosampling block for robotic sampling. Minimum volume 500 µL
Robot sampling from 2 mL tubes	Nucleic acid extracts stored in 2 mL tubes are loaded on the Autosampling block for robotic sampling. Minimum volume 40 µL
Robot sampling from 1.5 mL flip-cap tubes	Nucleic acid extracts are stored in 1.5 mL flip-cap tubes and loaded on the Autosampling block for robotic sampling.
Robot sampling from 96 well plate samples 1-24 (rows or columns)	Nucleic acid extracts are stored in a 96-well plate and placed in the Autosampling block section for robotic sampling of wells 1 - 24. Minimum volume 40 µL
Robot sampling from 96 well plate samples 25-48 (rows or columns)	Nucleic acid extracts are stored in a 96-well plate and placed in the Autosampling block section for robotic sampling of wells 25 - 48. Minimum volume 40 µL
Robot sampling from 96 well plate samples 49-72 (rows or columns)	Nucleic acid extracts are stored in a 96-well plate and placed in the Autosampling block section for robotic sampling of wells 49 - 72. Minimum volume 40 µL
Robot sampling from 96 well plate samples 73-96 (rows or columns)	Nucleic acid extracts are stored in a 96-well plate and placed on the Autosampling block section for robotic sampling of wells 73 - 96. Minimum volume 40 µL

8. INTERPRETATION OF RESULTS

WARNING: No IVD performance claims are made for this product. Any user changes (i.e., "Reject" or "Confirm" a result) will be clearly indicated in the Analysis Report.

The cycling curves and the melt curves of a run are displayed in the MT Analysis Software. Based on predefined parameters, the software will call the target as 'Present', 'Check 'or blank (not detected). **Note** that multiple infections are possible. Molecular target concentrations, expressed as arbitrary units, are calculated relative to the internal control SPIKE, which amplifies a known amount of target molecules. As the concentration of SPIKE is not measured these values are in arbitrary units. Furthermore, in some panels of assays the relative concentration of each target may be inferred from the normalised percent (displayed in parentheses after the 'Present 'call).

For further details on analysis of results, please refer to the Highplex IFU (**Section 11. Interpreting Software Results**).

9. CONTROLS

9.1 POSITIVE CONTROL

CONTROL +

It is recommended that positive controls be included in every run. Please refer to individual laboratory procedures. The failure of a positive control should lead to the reassessment of any negative result obtained since the last control was run.

The Synthetic Positive Controls for Faecal Panels (REF 91031) contains all targets for Faecal Bacteria and Parasites 12-well.

The Synthetic Positive Controls IFU must be read before using this product.

9.2 NEGATIVE CONTROL

It is recommended that a negative control be run according to the individual laboratory procedures. Amplification of the negative control indicates contamination from the environment (e.g., from handling during set up or spillages of sample material on the MT Processor deck). In this case, the surface of the MT Processor deck (including the thermal cycler cover) should be wiped with a non-corrosive nucleic acid denaturing reagent (e.g., DNA-OFF™) and then UV treated. The relevant samples should be retested. DO NOT USE BLEACH TO CLEAN THE INSTRUMENT.

9.3 SAMPLE ADEQUACY AND HUMAN DNA CONTROL

The Sample Adequacy Control assay targets a human DNA reference gene to indicate the presence of human DNA in the nucleic acid extract or direct sampling specimen. This assay is not intended to work with faecal samples due to the inconsistent and often degraded nature of human DNA in such samples. Except for faecal samples, if both the assay and sample adequacy control call negative, a new sample should be collected. If the assay calls positive and the sample adequacy calls negative, the sample is positive. For faecal samples, no conclusion can be drawn from a negative sample adequacy result.

9.4 SUITABLE NUCLEIC ACID CONTROL

It is the user's responsibility to ensure a suitable nucleic acid extraction procedure is in place. It is recommended a known positive control be included per extraction run. The Synthetic Positive Controls for Faecal Panels (REF 91031) can be used for a DNA control.

If the nucleic acid extraction control is not detected, the negative results cannot be relied upon. It is recommended that any sample with a negative nucleic acid extraction control should be re-collected and re- extracted if appropriate, and analysis repeated.

9.4 SAMPLE INHIBITION AND INSTRUMENT FUNCTION CONTROL

SPIKE is a completely artificial sequence that is present in Step 1 Tubes to monitor sample inhibition and instrument performance. SPIKE has been designed to have no cross-reactivity with diagnostic targets or assays. If SPIKE is shown to be inhibited, then this suggests that the sample contained inhibitory substances, or that the reaction conditions are suboptimal. In this case a negative result cannot be relied upon and it is recommended that the sample should be re-extracted if appropriate, and analysis repeated. For further details on analysis of SPIKE, please refer to the Highplex IFU (**Section 9.2 Verification of Instrument Function and Sample Inhibition**).

10. PERFORMANCE CHARACTERISTICS

10.1 REPRODUCIBILITY AND REPEATABILITY

The reproducibility of the assays on the Highplex was assessed by testing five samples on three batches across three days (with each batch tested once per day) and three systems with three operators. The coefficient of variation (c_V) for the resulting mean cycle take-off values (Ct values) for each of the samples were calculated. It was found that the Ct values for all samples at all concentrations were highly reproducible with c_V values ranging from 2.08% to 4.86%, averaging 3.40%.

The repeatability of the assays on the Highplex was assessed by testing five samples on three batches with one batch tested three times each day on one system. It was found that the Ct values for all samples at all concentrations were highly repeatable with c_V values ranging from 2.26% to 5.11%, averaging 3.84%.

The low c_V from these studies provides evidence that the Highplex is suitably precise for IVD use.

10.2 INTERFERING SUBSTANCES

A range of exogenous and endogenous substances including those expected to be found in blood were tested for potential PCR interference. Minimal or no interference was seen due to the presence of any one of the substances tested.

The presence of the internal control, SPIKE, in all AusDiagnostics panels controls for possible PCR interference in each sample.

For further details on interfering substances, please refer to the Highplex IFU (**Section 13.1 Interfering Substances**).

10.3 ANALYTICAL SPECIFICITY

Pathogens that are likely to be present in the specimen types used during the clinical validation were tested for cross-reactivity with the assays in this product. No cross-reactivity was seen.

10.4 ANALYTICAL SENSITIVITY

The limit of detection (LoD) was determined using serial dilutions of plasmids, with a multiplexed Step 1 amplification and 16 replicates per dilution tested. The LoD was determined to be the lowest concentration that showed 100% target amplification. Ranges are used when samples with higher concentrations were not detected, contrary to 100% amplification of samples with lower concentrations. The LoDs calculation is given as either copies per 10 µL sample or copies per mL of the original sample. The sample copies/mL calculation is based on 100% efficient nucleic acid extraction that concentrates a 200 µL sample into a 50 µL eluate.

Assay	LoD (copies/ 10 µL)	LoD (copies/ mL)
Salmonella	45-90	1125 - 2250
Shigella	33	825
Campylobacter	17	425
E.coli O157	16 - 78	400 - 1950
C.diff toxin B	36 - 71	900 - 1775
C.diff toxin A	51 - 82	1275 - 2050
Y.enterocolitica (Yersinia assay)	4-5	100-125
Y.pseudotuberculosis (Yersinia assay)	10 - 20	250 - 500
Shiga Toxin 1	51	1275
Shiga Toxin 2	9 - 19	225 - 475
Cryptosporidium	19 - 93	475 - 2325
Giardia	14 - 70	350 - 1750
E.histolytica	2-25	50-625

10.5 CLINICAL PERFORMANCE USING NUCLEIC ACID EXTRACTS

The clinical performance for the targets used in this product were assessed by multiple clinical laboratories in Australia, New Zealand and the United Kingdom. Each institution's alternative method was considered the reference method for this assessment.

Assay	SENSITIVITY % (95% CI)	SPECIFICITY % (95% CI)
C.difficile	97.7 (93.1-99.4)	99.6 (98.6-99.9)
tcdA*	100.0 (From 17 positive samples)	100.0 (92.0-100.0)
Campylobacter	100.0 (90.2-100.0)	100.0 (97.8-100.0)
Salmonella	100.0 (93.0-100.0)	100.0 (98.8-100.0)
Shigella	98.3 (89.7-100.0)	99.2 (98.8-99.5)
E.coli O157*	100.0 (From 10 positive samples)	100.0 (99.2-100.0)
Shiga toxin 1	87.0 (69.2-95.8)	100.0 (98.9-100.0)
Shiga toxin 2	97.2 (83.8-99.9)	100.0 (98.8-100.0)
Y.enterocolitica	93.3 (86.8-96.9)	100.0 (99.2-100.0)
Y.pseudotuberculosis*	80.0 (From 4 positive samples)	100.0 (99.3-100.0)
Crypto	97.8 (91.7-99.6)	99.8 (99.5-99.9)
E.histolytica	91.3 (70.5-98.5)	100.0 (98.1-100.0)
Giardia	98.6 (94.3-99.7)	99.6 (99.2-99.8)

* These assays have been validated with <20 confirmed positive samples. During primer design for each assay, bioinformatics analysis is conducted whereby the target sequence of the primers is analysed in all publicly available sequences. This analysis is based on published research and in-house studies on the importance of any mismatches in primer sequence on the efficiency of qPCR. This provides evidence that AusDiagnostics assays should detect all specified targets, and therefore assays validated with a low number of confirmed samples can be relied upon.

11. LIMITATIONS OF THE PROCEDURE

- This product is only for use by suitably trained personnel in qualified laboratories.
- Faecal Bacteria and Parasites 12-well Panel performance has only been established on AusDiagnostics Highplex.
- The assays in this product do not provide a quantitative value for the pathogen(s) in the sample.
- The performance of the test has been evaluated for use with human specimen material only.
- The performance of this test has only been validated with nucleic acid extracts from the following specimen types: faecal samples and culture
- The performance of this test has not been established for patients without symptoms of gastrointestinal illness.
- The performance of this test has not been established for immunocompromised individuals.
- The Faecal Bacteria and Parasites 12-well panel is designed to measure specific nucleic acid sequences. Therefore, a negative result does not exclude the possibility that an unusual sequence variant is present.
- The Sample Adequacy control cannot be relied upon for faecal samples due to the inconsistent and often degraded nature of human DNA in such samples.
- The detection of nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation (using manufacturer of specimen collection devices instructions). Failure to follow proper procedures can lead to incorrect results.

12. TECHNICAL ENQUIRIES

Further instructions and troubleshooting can be found in the Highplex IFU (**Section 14. Troubleshooting**).

For assistance or if any issues recur, please contact AusDiagnostics.
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13. ACKNOWLEDGEMENTS

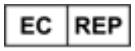
Edited by: J.Rosendale
Approved by: J.Haydar

14. GLOSSARY

The following symbols can be found on AusDiagnostics Faecal Bacteria and Parasites 12-well Panel components or throughout these Instruction for Use (IFU). Use the definitions below as a guideline to interpret the symbols.

14.1 SYMBOLS

Where relevant, symbols are taken from ISO 15223-1:2021 Medical devices - Symbols to be used with medical devices labels, labelling and information to be supplied.

	Manufacturer
	Authorised representative in the European Union
 or REF	Catalogue Number
 or Ver	Version number
LOT	Lot/Batch code
GTIN	This product has been assigned a unique Global Trade Item Number.
	Indicates that the relevant product is intended for in vitro diagnostic use in Australia and is included on the Australian Register of Therapeutic Goods (ARTG).
	Indicates that the relevant product is intended for in vitro diagnostic use in the European Economic Area and is compliant with the European IVD Directive 98/79/EC.
	**WARNING**. Please read the indicated section carefully.
	Please consult the identified instructions for use before use
	Do not re-use
	Storage temperature range (upper and lower limit)
	Storage temperature range (upper limit only)
	Positive Control
	Expiry date (yyyy-mm-dd)
DEVICE FOR PERFORMANCE STUDY ONLY (PSO)	The device is intended for performance study only. The device is not IVD marked or intended for IVD purposes.
DEVICE FOR RESEARCH USE ONLY (RUO)	The device is intended for research use only. The device is not IVD marked or intended for IVD purposes.
WARNING	Indicates a statement that alerts users about a situation that, if not avoided, could result in hazards or other serious adverse consequences from the use of the device
IMPORTANT	Indicates a statement that alerts the user to special care or special activities necessary for the safe and effectives use of the device

Note	Indicates additional information

14.2 DEFINITIONS

IMPORTANT: Indicates a statement that alerts the user to special care or special activities necessary for the safe and effectives use of the device

Note: Indicates additional information

Operator: The individual who is interacting with the Highplex system.

PANEL OF ASSAYS: A set of panel components that are intended to be used together to detect a specific group ("panel") of targets. Panel components include Step 1 Tubes and Step 2 Plates.

PANEL-SPECIFIC IFU: Instructions for Use (IFU) which contain information specific to a panel.

PRIMERS: Short synthetic oligonucleotides specifically designed to bind to and amplify specific gene sequences under conditions provided during PCR.

PSO: For performance study only. Clinical performance has not yet been established.

A RUN: All steps from starting the MT Assay Setup Software (see Section 7.3 Run the processor) to generation of the MT Analysis file is considered "a run"

RUO: For research use only. No clinical performance claims are made.

TARGET: A gene or sequence that primers are designed to hybridise to.

15. DISCLAIMER

AusDiagnostics does not warrant or guarantee that its products are merchantable or satisfactory for any particular purpose, and there are no warranties, express or implied, to such effect. AusDiagnostics will not be liable for any incidental, consequential or contingent damages involving the use of its products. AusDiagnostics 'responsibility is limited to replacement of items ordered only.

AusDiagnostics reserves the right to discontinue or change specifications, products, services, or models at any time without incurring obligations.

In no event shall AusDiagnostics be responsible for failures, errors, or other liabilities resulting from customers 'noncompliance with the procedures and precautions outlined herein or as a result of pathogen variants which were not sequenced at the time of assay design.

16. REFERENCES

1. Parliament, E., Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (Text with EEA relevance), in 1272. 2008.
2. Stanley, K.K. and E. Szewczuk, Multiplexed tandem PCR: gene profiling from small amounts of RNA using SYBR Green detection. Nucleic Acids Res, 2005. 33(20): p. e180.
3. England, P.H. Investigation of faecal specimens for enteric pathogens. 2015 [cited 2015 September]; Available from: https://www.gov.uk/government/publications/smi-b-30-investigation-of-faecal-specimens-for-enteric-pathogens.
4. England, P.H. Identification of Shigella species. 2015 [cited 2015 September]; Available from: https://www.gov.uk/government/publications/smi-id-20-identification-of-shigella-species.
5. Cheng, A.C., J.R. McDonald, and N.M. Thielman, Infectious diarrhea in developed and developing countries. J Clin Gastroenterol, 2005. 39(9): p. 757-73.
6. Dasti, J.I., et al., Campylobacter jejuni: a brief overview on pathogenicity-associated factors and disease-mediating mechanisms. Int J Med Microbiol, 2010. 300(4): p. 205-11.
7. Prevention, C.f.D.C.a. Reports of Selected E.coli Outbreak Investigations. [cited 2015 September]; Available from: www.cdc.gov/ecoli/outbreaks.html.
8. Pituch, H., et al., Hospital-based Clostridium difficile infection surveillance reveals high proportions of PCR ribotypes 027 and 176 in different areas of Poland, 2011 to 2013. Euro Surveill, 2015. 20(38).
9. Gerding, D.N., et al., Clostridium difficile binary toxin CDT: mechanism, epidemiology, and potential clinical importance. Gut Microbes, 2014. 5(1): p. 15-27.
10. Butler, T. Yersinia psuedotuberculosis. [cited 2018 April]; Available from: http://www.antimicrobe.org/b265.asp.
11. England, P.H. Identification of Vero cytotoxin-producing Escherichia coli including Escherichia coli O157. 2015 [cited 2015 September]; Available from: https://www.gov.uk/government/publications/smi-id-22-identification-of-escherichia-coli-o157.
12. Cherla, R.P., S.Y. Lee, and V.L. Tesh, Shiga toxins and apoptosis. FEMS Microbiol Lett, 2003. 228(2): p. 159-66.
13. Medema, G., et al., Risk assessment of Cryptosporidium in drinking water. World Health Organization, 2009: p. 143.
14. England, P.H. Investigation of specimens other than blood for parasites. 2014; Available from: https://www.gov.uk/government/publications/smi-b-31-investigation-of-specimens-other-than-blood-for-parasites.
15. Feng, Y. and L. Xiao, Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. Clin Microbiol Rev, 2011. 24(1): p. 110-40.
16. Fotedar, R., et al., Laboratory diagnostic techniques for Entamoeba species. Clin Microbiol Rev, 2007. 20(3): p. 511-32, table of contents.

17. DOCUMENT HISTORY

This document is the third IFU for Faecal Bacteria and Parasites 12-well.

Document ID of IFU	Date of Change	Changes
25041-r07	10/02/2023	Update of storage and handling instructions for Tandemplex® Step 1 tubes
25041-r06	31 Mar 2022	Specificity of Salmonella assay. Various formatting and corrections
25041-r05	23 Nov 2020	EC Representative details updated. Contents of step 2 box updated. Suitable nucleic acid extraction methods updated. Analytical sensitivity and specificity updated
25041-r04	14 May 2020	New USA office address. Added EMAG. Updated clinical performance.
25041-r03	06 Mar 2019	New product released with updated E.histolytica and Yersinia assays. Product CE-IVD marked and listed in the ARTG.
25041-r02	24 Aug 2018	Updated E.Histolytica and Yersinia assays
25041-r01	01 July 2018	Original document.