

### Example of Results Generated by PCR Marker Analysis for Twenty Different Water Samples

#### Results of PCR marker analysis:

Bold conclusion is the dominant or major source. Interpretations assume fresh faecal pollution, aged or partially treated faeces may have reduced levels of some source indicative markers.

ESR Number	General faecal marker-GenBac3	Human-BacH	Human-BiADO	Human-HumM3	Ruminant-BacR	Canine-DogBac	Avian-GFD	Seagull-gull2	Conclusion
CMB130xx1	Very strong positive	Present (high levels)	Present (high levels)	Present	ND	Present	ND	NA	<b>Human</b> Dog
CMB130xx2	Very strong positive	Present (high levels)	Present	Present	ND	ND	ND	NA	<b>Human</b>
CMB130xx3	Very strong positive	Present	Present	ND	ND	ND	Present	NA	Human Avian
CMB130xx4	Very strong positive	Present	Present	ND	ND	Present	Present	NA	Human Dog Avian
CMB130xx5	Very strong positive	Present	Present	Present	Present (Up to 10%)	ND	ND	NA	<b>Human</b> Ruminant
CMB130xx6	Very strong positive	Present	Present	Present	Present (>10%)	Present	Present	NA	<b>Human</b> Ruminant Dog Avian
CMB130xx7	Very strong positive	Present (high levels)	Present	Present	Present (~50%)	Present	ND	NA	<b>Human</b> <b>Ruminant</b> Dog
CMB130xx8	Very strong positive	Present	Present	Present	ND	ND	Present	NA	<b>Human</b> Avian
CMB130xx9	Extremely strong positive	Present (high levels)	Present (high levels)	Present (high levels)	ND	ND	ND	NA	<b>Human</b>
CMB130x10	Very strong positive	Present (high levels)	Present (high levels)	Present	Present (<1%)	Present	ND	NA	<b>Human</b> Dog

<b>ESR Number</b>	<b>General faecal marker-GenBac3</b>	<b>Human-BacH</b>	<b>Human-BiADO</b>	<b>Human-HumM3</b>	<b>Ruminant-BacR</b>	<b>Canine-DogBac</b>	<b>Avian-GFD</b>	<b>Seagull-gull2</b>	<b>Conclusion</b>
CMB130x11	Very strong positive	Present (high levels)	Present (high levels)	Present	ND	ND	ND	NA	<b>Human</b>
CMB130x12	Very strong positive	ND	ND	ND	Present (Up to 1%)	ND	Present	NA	Ruminant Avian
CMB130x13	Very strong positive	Present	ND	ND	Present (Up to 100%)	ND	ND	NA	<b>Ruminant (Up to 100%)</b>
CMB130x14	Very strong positive	Present (high levels)	Present (high levels)	Present	Present (Up to 5%)	Present	Present	NA	<b>Human</b> Ruminant Dog Avian
CMB130x15	Strong positive	ND	ND	ND	Present (Up to 50%)	ND	ND	NA	<b>Ruminant</b>
CMB130x16	Weak positive	ND	Present	ND	ND	ND	Present	Present	<b>Avian</b>
CMB130x17	Strong positive	Present	Present	ND	ND	ND	ND	NA	<b>Human</b>
CMB130x18	Weak positive	ND	ND	ND	ND	ND	Positive	Present	<b>Avian</b>
CMB130x19	Weak positive	ND	ND	ND	ND	ND	ND	NA	No source identified
CMB130x20	Very strong positive	Present	Present	Present	ND	Present	ND	NA	<b>Human</b> Dog

Abbreviations:

ND = sample was analysed, but the determinant was not detected.

NA = sample was not analysed for this determinant.

### Faecal Source - PCR Marker Assay Specificity

The specificity of the assays, as tested against known faecal samples is shown in the table below. These assays have all been tested against at least 200 known faecal samples.

Target group - Assay abbreviation	Recommended	Sensitivity	Detected in faeces from:	Low level non-specificity	Negative in faeces from:	Microbial target
General faecal marker - GenBac3	yes	High	Human, Cow, Sheep, Deer, Goat, Pig, Rabbit, Possum, Cat, Dog, Horse, Duck, Swan, Seagull, Geese, Chicken		(can be low in seagull and geese faeces)	Bacteroidales 16S rRNA
Human -BacH	Yes (1st preference for human)	Medium - most sensitive human assay	Human, Cat, Dog, Rabbit, Possum, Chicken, Goat	Swans, Geese, Seagulls, Pigs	Cow, Sheep, Deer, Horse, Duck	Bacteroidales 16S rRNA
Human -BiADO	Yes (2 <sup>nd</sup> preference for human)	Medium - less sensitive than BacH	Human, Seagulls	Possum, Dog, Duck, Swan	Cow, Sheep, Deer, Horse, Goat, Pig, Rabbit, Geese, Chicken Cat	Bifidobacterium adolescentis 16S rDNA
Human -HumM3	No	Low – only detected in highly polluted samples	Human, Possum, Rabbit	Horse, Goat, Dog, Seagull	Cow, Sheep, Deer, Pig, Duck, Swan, Geese, Chicken Cat	Bacteroidales cell wall protein
Ruminant -BacR	Yes	High	Cow, Sheep, Deer, Goat	Cat, some human municipal sewage, possum, geese	Human (individuals), Horse, Pig, Rabbit, Duck, Swan, Seagull, Chicken, Dog	Bacteroidales 16S rRNA
Bovine -CowM2	Only if highly polluted	Low	Cow	Deer, some human municipal sewage	Sheep, Goat, Horse, Pig, Human (individuals), Ducks, Swan, Geese, Seagulls, Cat, Dog, Possum, Rabbit	Bovine-specific faecal genetic markers
Sheep -Schill Sheep	Yes	Medium	Sheep	Goat, some human municipal sewage, Duck, Dog	Cow, Deer, Human (individuals), Swan, Geese, Seagull, Chicken, Horse, Cat, Pig, Possum, Rabbit	Cytochrome <i>b</i> of mitochondrial DNA

Target group - Assay abbreviation	Recommended	Sensitivity	Detected in faeces from:	Low level non-specificity	Negative in faeces from:	Microbial target
Canine -DogBac	Yes	High	Dog	Some human municipal sewage	Human (individuals), Cow, Sheep, Deer, Goat, Horse, Pig, Rabbit, Possum, Duck, Swan, Seagull, Geese, Chicken, Cat	Bacteroidales 16S rRNA
Avian -GFD	Yes	Medium	Duck, Swan, Seagull, Geese, Chicken		Human, Cow, Sheep, Deer, Horse, Goat, Pig, Rabbit, Possum Cat, Dog	Avian-specific faecal 16S rRNA
Avian -E2	Only if highly polluted	Low	Duck	Swan, Seagull, Geese, Chicken, Pig	Human, Cow, Sheep, Deer, Horse, Goat, Rabbit, Possum Cat, Dog	Desulfovibrio-like species 16S rRNA
Seagull -gull2	Only if highly polluted	Low	Seagull	None	Duck, Swan, Geese, Chicken, Human, Cow, Sheep, Deer, Horse, Goat, Pig, Rabbit, Possum Cat, Dog	Catellibacterium marimammalium 16S rRNA

- General faecal marker -GenBac3 results are reported on a semi-quantitative scale from Extremely Strong Positive down to Very Weak Positive.
- Ruminant -BacR is reported as a percentage of the GenBac3.
- All other assays are reported as present or not detected. Because of the varying sensitivity between assays we consider it may be misleading to use a semi-quantitative scale for reporting. For example, a 'strong positive' in the CowM2 assay and a 'strong positive' in the canine DogBac assay may not represent the same level of avian and dog faecal source.

In several marker assays "human municipal sewage" is detected at low levels. Because of the nature and source of municipal sewage we cannot be certain that it is a pure human source. The positive result seen in these assays may in fact be a true positive for the target marker.

Specificity testing of sources is an on-going activity. If testing other than listed in the above table is desirable, please enquire of any of the staff listed. Note that other markers assays will not have been tested against this range of known faecal samples.

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