City of Santa Fe

Agenda

CITY CLERK'S OFFICE
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SERVED BY Melissa McDonald
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Santa Fe River Commission Agenda
Thursday, December 14, 2017 (Round House Room), 6 pm to 8 pm
City Offices at the Market Station Building at the Railyard
500 Market Street, Suite 200, Santa Fe, NM
505-955-6840

1. **ROLL CALL**
2. **APPROVAL OF AGENDA**
3. **APPROVAL OF MINUTES FROM NOVEMBER 9, 2017**

4. **COMMUNICATION FROM OTHER AGENCIES /COMMITTEES**
   a. **SF Watershed Report (Andy Otto)**

5. **INFORMATION/DISCUSSION/ACTION:**
   a. **Information Item: Water Quality Testing for Santa Fe River, preliminary E.coli results (Staff)**
   b. **Discussion Item: Scoop-the-Poop initiative (Staff)**
   c. **Discussion Item: Santa Fe River Living River Sub-Committee Report (John Buchser)**
   d. **Discussion Item: Review 2018 River Commission Goals (John Buchser)**

6. **MATTERS FROM COMMISSIONERS**

7. **MATTERS FROM STAFF – 2018 meeting schedule reminder, SF River Fund Report, EPA pilot project, project status report**

8. **CITIZENS' COMMUNICATION FROM THE FLOOR**

9. **SUB-COMMITTEE BREAKOUT SESSION**

10. **ADJOURN**

Next Scheduled River Commission Meeting is January 11, 2018
Captions & Packet Material are due by 10 am on January 3, 2018
Persons with disabilities in need of accommodations,
Contact the City Clerk's office at
(505) 955-6521 five (5) working days prior to the meeting date.

Santa Fe River Commission
INDEX
December 14, 2017

Santa Fe River Commission
MINUTES
Thursday, December 14, 2017
6:00 pm to 7:50 pm

1. CALL TO ORDER

The Santa Fe River Commission meeting was called to order at 6:10 pm by Acting Chair Anna Hansen at the City Offices at the Market Station Building at the Railyard 500 Market Street, Roundhouse Meeting Room, Santa Fe, NM.

No quorum as reflected below.

2. ROLL CALL

PRESENT:
Anna Hansen, Acting Chair
Zoe Isaacson
Phil Bové

NOT PRESENT/EXCUSED:
John Buchser, Chair
Emile Sawyer
F.M. Patorni
Luke Pierpont
Dale Doremus
Jerry Jacobi

OTHERS PREENT:
Melissa McDonald, Staff Liaison
Alan Hook, City Staff
Raquel Baca-Thompson, Santa Fe Watershed Association
Aaron Kauffman, Santa Fe Watershed Association
Bob Findling, Nature Conservancy
Debbie Garcia for Fran Lucero, Stenographer

3. APPROVAL OF AGENDA

MOTION: No action, lack of quorum.

4. APPROVAL OF MINUTES: November 9, 2017

MOTION: No action, lack of quorum.

5. COMMUNICATION FROM OTHER AGENCIES /COMMITTEES

a. Santa Fe Watershed Association, Raquel Baca-Thompson

Raquel reported that the Sierra Club is going to start working with the Santa Fe Watershed Association on the vegetation fitting on the river, reaches to Alameda and St. Francis upstream. The adopt an arroyo program is moving along nicely; they have 6 sponsors and 6 steward teams. The signs are going up along the arroyos, thank you

to the city of Santa Fe they are working with Chris Ortiz and they are putting in the big posts and SF Watershed has started ordering signs. They also have one arroyo plan in place, they are working with Aaron Kaufman with Southwest Urban Hydrology. The plan is for Arroyo en Medio and it can be viewed on the Santa Fe Watershed website.

Ms. Hansen asked if they are doing any adoptions for arroyos in Santa Fe County. Ms. Baca-Thompson said that right now they do not have any arroyos in the county; they can and they will. We don't have any set reaches right now, but we will go in to any arroyo, so if it is in the county, yes, we can do that.

Ms. Hansen stated that she has some constituents who are complaining about an arroyo in the County so it might be a good idea if Ms. Baca-Thompson could go out and answer some questions for them. (Ms. Hansen will send contact information to Ms. Baca-Thompson for follow up by Keely and Aaron).

A question from Mr. Puglisi; what do you mean by adopt?

Ms. Baca-Thompson said that the Adopt an Arroyo program is the same as the Adopt a River Program, there are certain reaches of the river and they have particular sponsors that pay about $1000 a year to help maintain that reach. We get a steward team, volunteer groups or schools that go out – we required three times a year – they go out and they clean it.

Ms. McDonald said that it is an extension of the River program and eventually we will make it it's own program if we get enough arroyo's in the city. Ms. McDonald asked Raquel if they work with Santa Fe County on signs, they are working on signs and mentioned follow up would be good for signs. Contact: Maria Loehman from Planning at Santa Fe County.

Ms. Hansen expressed her thanks to the Santa Fe Watershed Association for the great holiday party.

6. INFORMATION/DISCUSSION/ACTION:

Nature Conservancy, Bob Findling

Provided information to the River Commission on a forestry treatment project they are involved with the Santa Fe Fire Department. It was an earlier phase of some thinning work on municipal property referring to the Santa Fe Canyon Reserve. (Exhibit A shows the areas that Mr. Findling made reference to in his presentation). Previously there has been some forest treatment work done on Aztec Springs Creek, which is attributor to the Santa Fe River, the confluence is near the Audubon Center just below Nichols. There was a nature conservancy burn together with the Fire Department. The prescription includes mostly Pinon – Juniper thinning to reduce fuel loads adjacent to the Water Shed. The terrain is too steep to permit thinning work with a macerator type device. This slope analysis, everything in orange exceeds 50% so very steep. There is a tremendous amount of fuel in these areas, some unauthorized habitation, mostly homeless people, generally seasonal, one person who lives there almost year round. There is not adequate security to close the area, it is essentially open and so you can remove the personal effects but you cannot prohibit or keep people from drifting up there who do not have a permanent habitation. There is a risk of wildlife fire in the vicinity. In the municipal treatments

the woody material is cut and slashed into small burn piles. The prescription requires that the burning only occurs when there is snow on the ground and that is problematic. This is the time of year with low temps, generally low winds, when the weather is favorable for conducting prescribed burns and we don't have snow. All week there were burn piles being burned without prescription. We anticipate using that prescription on the thinning work being conducted with the cooperation of the Fire Department on Nature Conservancy property. Approximately 150 acres will be treated (showed area on map), towards the ski basis. The thin slopes are above Nichols and the Municipal Water Facility was pointed out, showed the Aztec Springs and properties on Hyde Park. The grant that the Fire Department will serve as the Fiscal Agent for came through, it is federal money for non-federal land and forest treatment work. It came from State Forestry to the Fire Department and most of the thinning work will occur on conservancy property. If there were a fire in that area it would consume Hyde Park. Because of the extreme restriction from the first pile burning, the 80 acres that were treated previously, only 20 acres have the piles been burned. There are 60 acres of woody piles that need to be burned and eliminated. Unless the City Manager the directive on pile burning that probably is not going to happen this year. We do have a crew in Colorado that has done similar work where the piles are burned at the time they are cut. That is not something that has been tried here. Next phase of work on the conservancy property will be treated in that manner as a demonstration. The thinning prescriptions will be the same, pile burning will not be restricted to only circumstances when there is snow on the ground. It is anticipated that the work will begin next fall and it will be over 3-years. Somewhere between 30-50 acres per year over the 3-year term and hopefully at some point during that term there will be adequate snow to be able to deal with the burn piles on municipal property that haven't been addressed.

Ms. Hansen asked how much snow has to be on the ground?

Mr. Findling said adequate cover, 6" probably and no wind. The slope analysis was done by the Santa Fe Fire Department.

Ms. Hansen asked where is the homeless person living.

Mr. Findling said the individual resides (points out in the map where he lives). Ms. McDonald asked if someone would go up and let him know.

a. Water Quality Testing for Santa Fe River, preliminary e-coli results.

Haley introduced herself as the Intern who worked for the Water Department for 2 years, she is now doing her Masters at UNM in Geography. Basically they used a new technique in this research called genetic source tracking which normally you can identify contaminates e-coli but you don't know where that e-coli or bacteria came from. With this testing you are able to identify the genetic source. What they did is pick 5 locations off the Santa Fe River and Alameda and they tested for dog, human, beaver depending on the site. The general results right now show that if you have any type of contamination at any point along the river it is most likely going to be due to dogs, the second highest concentrations were human. Abien came up negative for a lot of the sites and we had beaver come up as present but not quantifiable. We have several pages that show the concentration. There was only a high concentration at the Paseo crossing for dogs.

Mr. Puglisi said that the project was limited due to funding and we came up with representative sites and the sheets show what we expected to find at those sites. This is not an inexpensive process; it is approximately $275-$300 per marker. Five locations with five markers are quite expensive. We did not have that much money available in our budget this year but we hope to do this next year. We only did one test during a storm with the runoff the next day. The qualitative report shows the results. We picked pretty heavy test sites except for Agua Fria. They are not using an actual count of organisms, they are using a relative comparison to come up with their data. They define these markers but when they say, low – medium – high, it is relative concentration. It was stated it is relative concentration to each of the sites, the data set not comparable. We are going to follow up to see if we could get percentages or some type of indicator on how these compared to the National Standard to what is good water quality. Haley said she will write up a full report in January and it will have the specifics on how they tested as well.

Allan stated that the State does sampling but it is a different scale, it is normally a probable number per 100 millimeters, I am wondering I that could be used in a comparable manner.

Mr. Puglisi said that what they were told was that they don't report markers in that way because the probable number is probably a colony count. Fecal bacteria forms colonies and the standard is formed on colony forming units. Example, the standard is 100 cfu and so they can count the colony but when they are comparing the bio markers they can tell you that 25% of the sample had dog markers but they can't tell you exactly how many there were. When you see a high concentration you know there had to be a lot of fecal from dogs to give you that high number, it is all relative.

Ms. Hansen stated that she was not surprised there are dogs but she is surprised there is human.

Staff did review the human report. Ms. McDonald stated this is interesting and hopes we can continue it through the MS4 permit they are going to want us to do more and more water quality testing. I am really happy that Haley and Alex did this project, it was good to get the baseline. If we could do in the spring or summer, we could have a good indication.

All of the e-coli was low based on the tests. Ms. McDonald said there was more dog poop than human. It is all relative. There could be more bird contamination but the human was low and less present.

Ms. Hansen asked how far down Agua Fria did they go or are they going? Mr. Puglisi said they are right below the crossing.

Ms. Hansen stated that there are a lot of homeless people that live around there and there are septic tanks. We have septic issues and we also have cesspools.

Mr. Puglisi shared with the Canyon Road Neighborhood Association members that in light of these values it would be good to go back and look at nitrate concentration at these sites. Because if it is sewage contributing you are going to find higher nitrates.

Beaver: Tested on Cerro Gordo where we know we have Beavers, and it was low. Ms. Hansen asked if we know we have a beaver population and it is low, what does that mean? Mr. Puglisi said part of the reason they chose that location is because it was one of the first sites for NMEDs assessment. We didn't choose it just because of beavers, it is one of the upper most sampling site on Canyon Road. You would expect the Cerro Gordo to be somewhat pristine since it is coming out of the watershed and the preserve, but the birds and the beaver, you wouldn't expect the river to exceed the e-coli standard for the Santa Fe River, for cold water fishery but it does, it is impaired at that site. We wanted Haley help us demonstrate to NMED that this is a pristine site and the river is already impaired and it hasn't even gone through the municipal section of town yet.

Bird is entirely in the county. (Exhibit included) One of the five was not quantifiable.

Last was done on Agua Fria, i.e., horses. Mr. Puglisi said that when they go back they will try to expand the sub-set to include all of these plus others we will know that will most likely be present. That gets really expensive, in the future the way we can use this is for storm water and for compliance with water quality standards. We can make an argument with the Council and Ms. McDonald has some money in storm water quality; that a much larger study needs to be done and we have the person to do it for us. We should really do that to decide on do we need to do more investigation say on the source of human contamination, fecal contamination, do we have it warranted where we can do more septic tank nitrate sampling or do we have areas where appropriate samplings and see if things have changed say the dogs which is a big contributor.

Allan asked if the State has offered to do a match. Ms. McDonald hasn't approached them but she will ask them. Mr. Puglisi said there is a grant that he sent information to staff that can be used for this purpose. It is for target streams that have been impaired that have had TMDLs (Total Maximum Daily Loads) developed. I asked them if something like this would qualify under their program and they said yes, but, I was going to review their application requirements.

Ms. McDonald said all of this good information for us, maybe there is a map that shows where there are more pollutants, and with the Scoop the Poop initiative that will be very helpful for identifying areas. We talk about having TMDL's and we don't mark the river do not swim here, but the river is not safe to swim in, after a storm especially. We want to look at normal living river flow. What do we see then, what is coming in from surrounding areas. If it is a normal flow regime and we are seeing lower numbers, at least we know the river is safe and not a storm event. If you look at the samples many were done after storm events. Ms. McDonald reached out to Haley and asked if she would like to help write a grant request for this purpose. Yes.

Mr. Puglisi said if they were to sample up at the Preserve where there are ducks and geese and we don't see it there, then we would be suspicious. The river has a good canapé.

Thank you to Haley for her work.

b. Discussion Item: Scoop the Poop Initiative

Ms. McDonald introduced Jane Larsen and Robin Hardin. They are with the Frank Ortiz Dog Park and they shared some of the work that they are doing.

Robin and Jane started the dog park project in February of 2017 and it resulted in them adopting the Frank Ortiz Dog Park. It is really a treasure to the city; the park combined with the open space is 130 acres, it is an unusual park for Santa Fe. It is the most trafficked park in Santa Fe. One of the things that motivated them, and the dog park project is a non-profit, was to make this park to be a cleaner park and to be a safer park. Those who have patronized the park for many, many years have know that the mentality has changed. At one time they didn't clean after their dogs, there were homeless people and there was a lot of dumping.

Ms. Hansen said that people dump garbage there, and this is over 45 years of living here. That needs to be looked in to.

Ms. Hardin stated that it wasn't managed as a landfill so therefore it doesn't fall in to a specific designation, so the city dumped there.

Ms. Hansen said that she will talk to Randall at Solid Waste, we need to be careful how we categorize it.

The good news is that for all intensive purposes it was a dump and it could never be built on so the dogs have the best real estate in the city. Robin and Jane want to see this park be cleaner and safer. One of the ways we see is to place signage on waste. We have encountered hostility from some people who don't want to see it as a park. We have been working with a local artist, Hilary Vermont who is a wonderful pet artist in town. She has come up with signage (samples were shown), some have to do with drive safely. We have 6 samples, they say clean trails is happy tails. Because we have had a fair amount of hostility from people who have been up there, we wanted it to not be threatening and to make it fun. Santa Fe is an art community. Melissa was thinking there could be one big sign along the river. The city doesn't like the work poop, scoop the poop so the signage would be a good visual piece.

Zoe asked if they had thought of where people would throw their poop, have you thought of trash bins.

The city will empty the bins in the Frank Ortiz Park, and they will give us colorful trash bins.

Zoe asked if the signs would be all through the trails.

They will only be in the Frank Ortiz Park vicinity. It was noted that when they are walking they scoop other people's poop to keep the park clean. The city cannot have trash bins where the city cannot get in to. The park is changing and people are working together. We have other objectives, we would like to raise money for water stations as we are non-profit, also possibly shade stations. Most people that go there want to pick up their poop, we didn't want signs that were intimidating. There are people that no matter what kind of sign you put there are not going to comply.

Ms. Hansen likes the signs and the message that is being projected.

There was a suggestion to add; no digging.

The message we want to get out is "we like the dog park".

Allan asked if they have tied in with the neighbors.

There is a fair amount of buffer, there is sort of a rim around it.

Ms. Hansen said she thinks it is great and we should try to find away to collaborate. The Acequia should also be considered, educating people that this is a health problem. It is hard to talk to people sometime.

Ms. McDonald said thank you, she is so glad to see how much they are already doing. We need to educate our community people so they understand that the dogs should not be playing in the water.

It was stated that it would be fun to have visual continuity through the city. The artist is one of the best dog artist in the city. Ms. McDonald said that it would be possible that the Watershed would like to collaborate. This is the start of creating a cohesive group to work on the signage project. Ms. McDonald asked Ms. Hansen if they would be willing to talk to meet with Maria Loehman and share some of this information on consistent messaging. Ms. Hansen also recommended that the ladies go to the county and collaborate with them as well.

Thank you so much for attending the meeting and providing this information.

Mr. Puglisi said that they have turned in a report to MVD for Ortiz Park, a lot of results are back and Phase I investigation is complete.

c. **Discussion Item: Santa Fe River Living River Sub-committee Report**
Report at next meeting.

d. **Discussion Item: Review 2018 River Commission Goals**
Report at next meeting.

7. **MATTERS FROM COMMISSIONERS**
None

8. **MATTERS FROM STAFF**
- 2018 Meeting Schedule Reminder, Santa Fe River Fund Report, EPA pilot project, project status report. EPA now has from the report at last month's packet, we came up with some goals. There are 6 goals that have been identified from the last meeting. From the goals they are asking us to get internal and external stakeholders who can communicate with EPA. They will meet with city staff and they will then need people to review it. Watershed has agreed to be on one of the Goal groups and Ms. McDonald will be reaching out to the River Commission members and the Water Conservation Committee and maybe Solid Waste. It is anticipated to have a lot of data to review, it will be taken to the community for feedback and there is a lot of modeling. The modeling is on GIS, one for water quality and water pollutant quality and we can look at where our flows are and then we can overlay it for review. They were also getting more detailed where sediment is. As it progresses, Ms. McDonald

will share more information. Ms. McDonald said they are working with Jesse Roach a local contact and with Rosemary Romero who will manage the public outreach.

- Santa Fe River Fund, budget office would like to report at meeting in January 2018.
- Ms. McDonald will be sending out a citywide calendar.
- Reminder: Elections will be conducted in January for the position of Chair.
- Reminder: Talking about looking at committee makeup in April, we need to think ahead on who we would want to invite to this committee and the terms of their appointment. It would be good to wait until after the Mayoral election.
- 2018 River Commission Goals: Included in packet – look at to discuss in 2018 to confirm if our goals are in line.
- The underpass is having their opening on December 18th at 1:30 pm.

9. CITIZENS' COMMUNICATION FROM THE FLOOR

10. SUB-COMMITTEE BREAKOUT SESSION – deferred to next meeting.

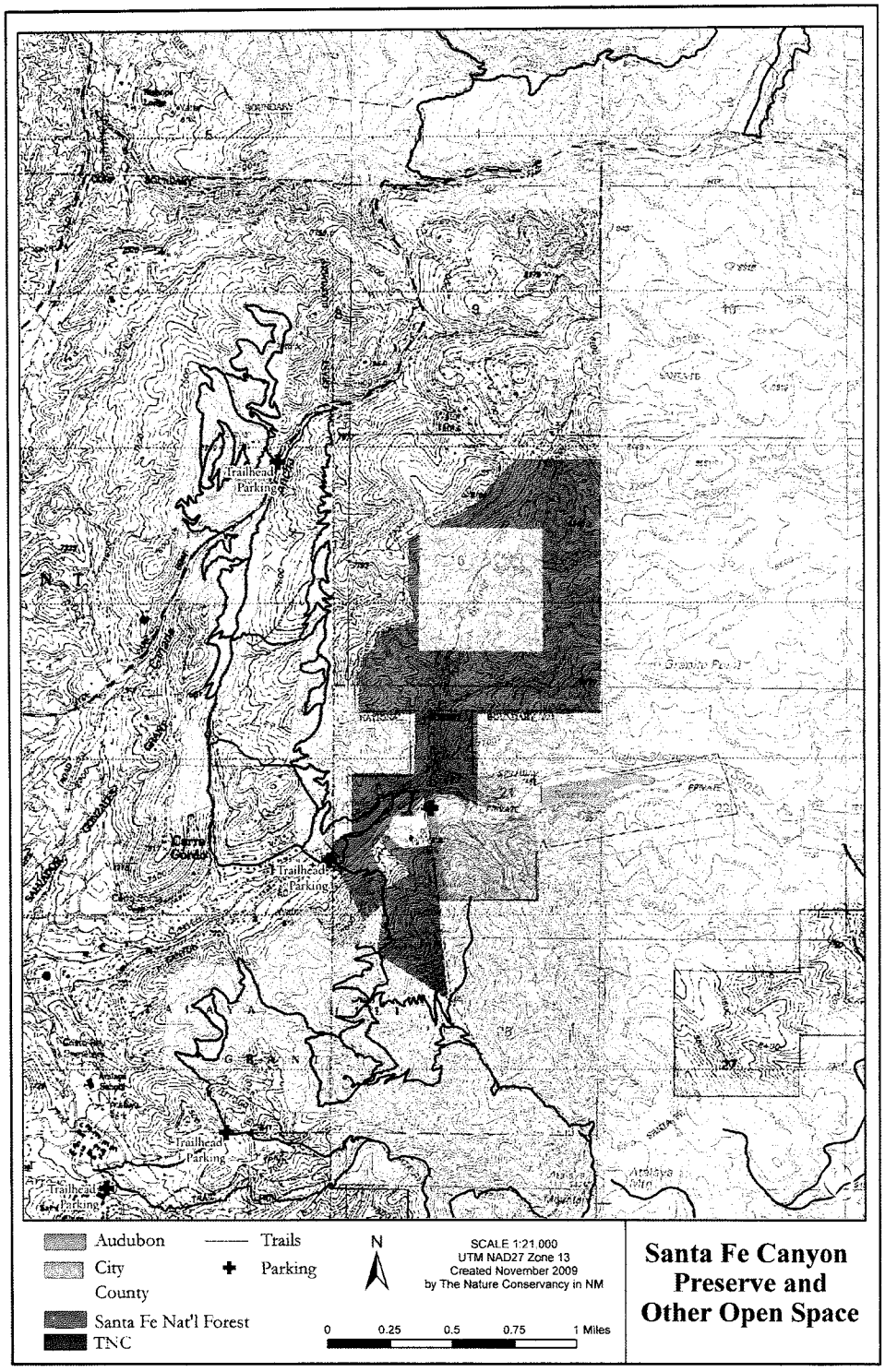
11. ADJOURN

There being no further business to come before the River Commission, the meeting was adjourned at 7:50 pm.

Signature Page:

~~**Anna Hansen Acting** **Chair**~~ John R. Buchser

Fran Lucero, Stenographer


Trailhead Parking
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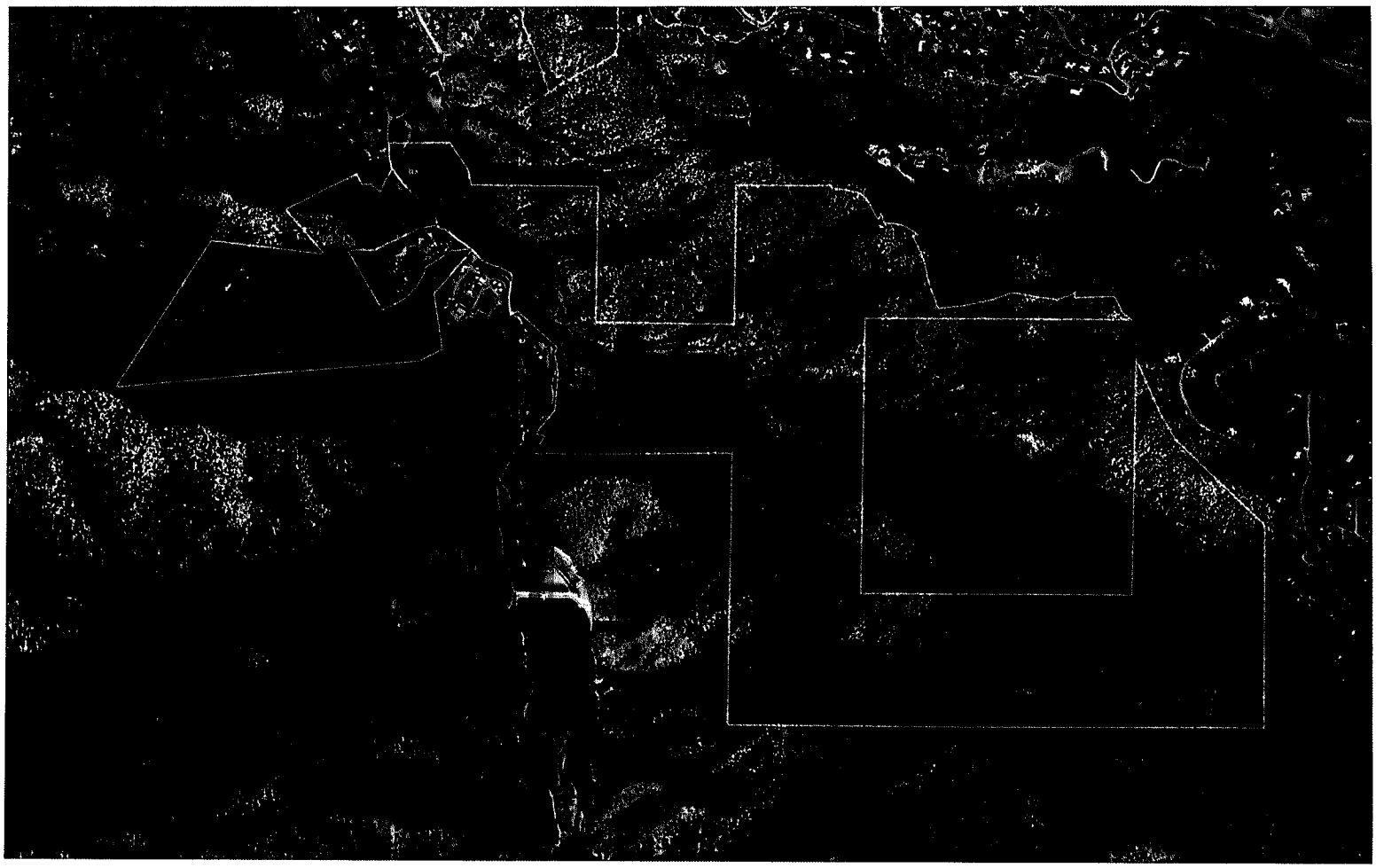
Trailhead Parking
Audubon
City
County
Santa Fe Nat'l Forest
TNC
Trails
Parking
N
SCALE 1:21,000
UTM NAD27 Zone 13
Created November 2009
by The Nature Conservancy in NM
0 0.25 0.5 0.75 1 Miles
Santa Fe Canyon Preserve and Other Open Space

Exhibit A-1



Client Reference	Analysis Requested	SM Number
# 1 Cerro Gordo	Human Bacteroidetes ID: Dorei	SM-7I29001
# 2 Patty Smith Park	Human Bacteroidetes ID: Dorei	SM-7I29002
# 3 Paseo	Human Bacteroidetes ID: Dorei	SM-7I29003
# 4 Guadalupe	Human Bacteroidetes ID: Dorei	SM-7I29004
# 5 Agua Fria	Human Bacteroidetes ID: Dorei	SM-7I29005
# 1 Cerro Gordo	Beaver Fecal ID	SM-7I29006
# 1 Cerro Gordo	Bird Fecal ID	SM-7I29007
# 2 Patty Smith Park	Bird Fecal ID	SM-7I29008
# 3 Paseo	Bird Fecal ID	SM-7I29009
# 4 Guadalupe	Bird Fecal ID	SM-7I29010
# 1 Cerro Gordo	Dog Bacteroidetes ID: Target 1	SM-7I29011
# 2 Patty Smith Park	Dog Bacteroidetes ID: Target 1	SM-7I29012
# 3 Paseo	Dog Bacteroidetes ID: Target 1	SM-7I29013
# 4 Guadalupe	Dog Bacteroidetes ID: Target 1	SM-7I29014
# 5 Agua Fria	Dog Bacteroidetes ID: Target 1	SM-7I29015
# 5 Agua Fria	Ruminant Fecal ID: Target 1	SM-7I29016

Exhibit B

Source Molecular Corporation
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

Revision 1.0
Effective Date 8/31/17

Beaver Fecal Quantification ID

Detection and quantification of the fecal associated Beaver gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-7I29006	# 1 Cerro Gordo	Beaver Fecal ID	DNQ	Detected

Source Molecular Corporation
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

Preliminary Interpretation of Beaver Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Beaver gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

	INTERPRETATION	
Sample ID	**Concentration of Beaver Fecal Pollution in Sample**	**Comment**
# 1 Cerro Gordo	**Low Concentration**	Low levels of Beaver fecal biomarker(s)

Laboratory Comments

Submitter: City of Santa Fe
Report Generated: October 26, 2017

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation
Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Beaver Fecal ID Theory Explanation

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[1] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Beaver Fecal ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[2,3,4,5,6] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in beaver. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in beaver[2,3,5,6] As such, these bacterial strains can be used as indicators of beaver fecal contamination.

One of the advantages of the Beaver Fecal ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve would indicate that the beaver *Bacteroidetes* gene biomarker is not present.

References

[1] Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy Microbial Source Tracking: Current Methodology and Future Directions. Appl. Environ. Microbiol. (2002) 68: 5796-5803.

[2] Bernhard, A.E., and K.G. Field (2000a). Identification of nonpoint sources of fecal pollution in coastal waters by using host -specific 16S ribosomal DNA genetic markers from fecal anaerobes. Applied and Environmental Microbiology, 66: 1,587-1,594.

[3] Bernhard, A.E., and K.G. Field (2000b). A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. Applied and Environmental Microbiology, 66: 4,571-4,574.

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[5] Fogarty, Lisa R., Voytek, Mary A.Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species Appl. Environ. Microbiol. 2005 71: 5999-6007.

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Bird Fecal Quantification ID

Detection and quantification of the fecal associated Bird gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-7I29007	# 1 Cerro Gordo	Bird Fecal ID	4.13E+03	Detected
SM-7I29008	# 2 Patty Smith Park	Bird Fecal ID	DNQ	Detected
SM-7I29009	# 3 Paseo	Bird Fecal ID	DNQ	Detected
SM-7I29010	# 4 Guadalupe	Bird Fecal ID	DNQ	Detected

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Preliminary Interpretation of Bird Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Bird gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

Sample ID	INTERPRETATION	
	Concentration of Bird Fecal Pollution in Sample	Comment
# 1 Cerro Gordo	Low Concentration	Low levels of Bird fecal biomarker(s)
# 2 Patty Smith Park	Low Concentration	Low levels of Bird fecal biomarker(s)
# 3 Paseo	Low Concentration	Low levels of Bird fecal biomarker(s)
# 4 Guadalupe	Low Concentration	Low levels of Bird fecal biomarker(s)

Laboratory Comments

Submitter: City of Santa Fe

Report Generated: October 26, 2017

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation
Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Bird Fecal ID™ Quantification

The genus *Helicobacter* is a group of gram-negative, microaerophilic bacteria that were initially classified under the *Campylobacter* genus prior to 1989. Since then, they have been reclassified into the genus *Helicobacter* after 16S rRNA sequencing differentiated them from other *Campylobacter* species. This group of bacteria typically have a spiral, curved or fusiform morphology with multiple flagella allowing them to rapidly maneuver in the intestinal mucous lining of their hosts. *Helicobacter* species colonize the gastrointestinal tract of mammals and birds and are shed in feces. There are approximately 20 strains of *Helicobacter*[1]. Certain strains, such as *Helicobacter pylori,* are pathogenic to humans causing chronic gastritis, peptic ulcers and stomach cancer.
The Bird Fecal Quantification ID™ service is designed around the principle that certain DNA sequences contained within strains of the *Helicobacter* genus are specific to wild birds. These *Helicobacter* sequences can be used as indicators of bird fecal contamination. Several species have been isolated from specific animal hosts such as *H. fennelliae* from humans, *H. hepaticus* from mice and *H. felis* from cats and dogs.[1]
The Bird Fecal Quantification ID™ service targets a bird-associated gene biomarker in *Helicobacter pametensis.*[2] The biomarker is present at different degrees in the feces of various birds including but not limited to gull, goose, chicken, pigeon and duck.
One of the advantages of the Bird Fecal Quantification ID™ service is that the entire population of *Helicobacter* of the selected portion of the water sample is screened. As such, this method avoids the randomness effect of selecting isolates off a petri dish.

Accuracy of the results is possible because the method uses qPCR DNA technology. qPCR simulataneously confirms and quantifies the bird-associated gene biomarker. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genome to be detected. This qPCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus.
Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve indicates that the bird-associated *Helicobacter* gene biomarker is not present.

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Dog Fecal Quantification ID

Detection and quantification of the fecal associated Dog gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-7I29011	# 1 Cerro Gordo	Dog Bacteroidetes ID: Target 1	4.54E+03	Detected
SM-7I29012	# 2 Patty Smith Park	Dog Bacteroidetes ID: Target 1	3.16E+04	Detected
SM-7I29013	# 3 Paseo	Dog Bacteroidetes ID: Target 1	1.13E+05	Detected
SM-7I29014	# 4 Guadalupe	Dog Bacteroidetes ID: Target 1	2.26E+04	Detected
SM-7I29015	# 5 Agua Fria	Dog Bacteroidetes ID: Target 1	2.14E+04	Detected

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Preliminary Interpretation of Dog Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Dog gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

Sample ID	INTERPRETATION	
	Concentration of Dog Fecal Pollution in Sample	**Comment**
# 1 Cerro Gordo	**Low Concentration**	Low levels of Dog fecal biomarker(s)
# 2 Patty Smith Park	**Moderate Concentration**	Moderate levels of Dog fecal biomarker(s)
# 3 Paseo	**High Concentration**	High levels of Dog fecal biomarker(s)
# 4 Guadalupe	**Moderate Concentration**	Moderate levels of Dog fecal biomarker(s)
# 5 Agua Fria	**Moderate Concentration**	Moderate levels of Dog fecal biomarker(s)

Laboratory Comments

Submitter: City of Santa Fe

Report Generated: October 26, 2017

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation
Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Dog Bacteroidetes "Quantification" ID™

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[1] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Dog Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[2,3,4,5,6] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in dog. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in dog.[2,3,5,6] As such, these bacterial strains can be used as indicators of dog fecal contamination.

One of the advantages of the Dog Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve. The absence of an amplification curve would indicate that the dog *Bacteroidetes* gene biomarker is not present.

References

[1] Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. (2002) 68: 5796-5803.

[2] Bernhard, A.E., and K.G. Field (2000a). **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Applied and Environmental Microbiology, 66: 1,587-1,594.

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[5] Fogarty, Lisa R., Voytek, Mary **A.Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. 2005 71: 5999-6007.

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Human Fecal Quantification ID

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-7I29001	# 1 Cerro Gordo	Human Bacteroidetes ID: Dorei	2.67E+02	Detected
SM-7I29002	# 2 Patty Smith Park	Human Bacteroidetes ID: Dorei	DNQ	Detected
SM-7I29003	# 3 Paseo	Human Bacteroidetes ID: Dorei	DNQ	Detected
SM-7I29004	# 4 Guadalupe	Human Bacteroidetes ID: Dorei	4.72E+02	Detected
SM-7I29005	# 5 Agua Fria	Human Bacteroidetes ID: Dorei	2.88E+03	Detected

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Preliminary Interpretation of Human Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

Sample ID	INTERPRETATION	
	Concentration of Human Fecal Pollution in Sample	Comment
# 1 Cerro Gordo	**Low Concentration**	Low levels of Human fecal biomarker(s)
# 2 Patty Smith Park	**Low Concentration**	Low levels of Human fecal biomarker(s)
# 3 Paseo	**Low Concentration**	Low levels of Human fecal biomarker(s)
# 4 Guadalupe	**Low Concentration**	Low levels of Human fecal biomarker(s)
# 5 Agua Fria	**Low Concentration**	Low levels of Human fecal biomarker(s)

Laboratory Comments

Submitter: City of Santa Fe

Report Generated: October 26, 2017

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation
Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Human Bacteroidetes ID™ Species: *B. dorei*

The **Human Bacteroidetes ID™ Species: *B. dorei*** service targets the species *Bacteroides dorei*. *B. dorei* is an anaerobe that is frequently shed from the gastrointestinal tract and isolated from human feces worldwide. It is a newly discovered species that is widely distributed in the USA.[1,2] The human-associated marker DNA sequence is located on the 16S rRNA gene of *B. dorei*.[3] The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its exceptional sensitivity and specificity. Internal validations have been conducted on hundreds of sewage, septage, human and animal host fecal samples collected from throughout the U.S and archived in the Source Molecular fecal bank. The marker has also been evaluated in both inland and coastal waters. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei* qPCR assay to be the top performing human-associated assay amongst those tested. The success and consistency of this marker in numerous studies around the world[1,3,4] makes the **Human Bacteroidetes ID™ Species: *B. dorei*** service the primary service for identifying human fecal pollution at Source Molecular.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[5] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The Human Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[3,5,6,7,8] Furthermore, certain strains of *Bacteroidetes* have been found to be associated with humans.[3,6] As such, these bacterial strains can be used as indicators of human fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve by the qPCR software. The absence of an amplification curve indicates that the *B. dorei* gene biomarker is not detected in the water sample because it is either not present or present at concentrations below the analytical detection limit.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as
Human Bacteroidetes ID™ Species: *B. stercoris*,
Human Bacteroidetes ID™ Species: *B. fragilis*, and
Human Bacteroidetes ID™ Species: *B. thetaiotaomicron*.

[1]Boehm, A., Fuhrman, J., Mrse, R., Grant, S. **Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California**. Environ Sci Technol. 2003 37: 673–680.
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[6] Bernhard, A., Field, K. **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Appl. Environ. Microbiol. 2000a 66: 1587-1594.

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Ruminant Fecal Quantification ID

Detection and quantification of the fecal associated Ruminant gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-7I29016	# 5 Agua Fria	Ruminant Fecal ID: Target 1	DNQ	Detected

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Preliminary Interpretation of Ruminant Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Ruminant gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: City of Santa Fe
Date Received: September 29, 2017
Report Generated: October 26, 2017

Sample ID	INTERPRETATION	
	Concentration of Ruminant Fecal Pollution in Sample	Comment
# 5 Agua Fria	Low Concentration	Low levels of Ruminant fecal biomarker(s)

Laboratory Comments

Submitter: City of Santa Fe

Report Generated: October 26, 2017

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Ruminant Fecal ID™ Quantification

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae* . This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (*i.e.Bacteroides*), but since the 1990's findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci.*[1] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci.* Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Ruminant Fecal Quantification ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[2,3,4,5,6] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in ruminants. Within these *Bacteroidetes*, certain genetic sequences in the *Bacteroides* and *Prevotella* genus have been found in ruminants.[7] As such, these bacterial strains can be used as indicators of ruminant fecal contamination.

One of the advantages of the Ruminant Fecal Quantification ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected. Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available and detected in real-time. Quantitative PCR (qPCR) adds a variant to the PCR process by inserting of a fluorescent probe within the primer set. This fluorescent probe serves as a molecular beacon for the quantification step. During each PCR cycle, quantitative PCR monitors the fluorescence emitted during the reaction. This is done in real-time during the first PCR cycles as a way to quantify the targeted gene. Absolute quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of plasmid DNA containing a known amount of the ruminant-specific biomarker. The Ruminant Fecal Quantification ID™ service uses qPCR to simultaneously confirm and quantify the ruminant- specific fecal *Bacteroidetes* genetic biomarker. This PCR technology avoids the cumbersome process of distinguishing DNA bands on a gel electrophoresis apparatus.

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