

Laboratory Sample Processing

Western Growers Association Pre-Harvest Product Sampling and Testing Workshop



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Certified Group

Laboratory Testing | Regulatory Consulting

Learning Objectives

- Understand how samples are accessioned upon arrival at the laboratory.
- Understand how samples are set for testing.
- Understand what detection methods are used and how they are performed.
- Understand the process for reporting out results.
- Understand the principles for evaluating and choosing a laboratory partner.



Background

What Is A Third-Party Laboratory?

- For-profit business that performs testing services for clientele.
- Clients request testing on products or on samples from production environments:
 - Product Release
 - Routine Monitoring / Process Control
- Basic Process:
 1. Client submits samples with requested tests
 2. The third party laboratory performs the tests
 3. Laboratory provides a certificate of analysis with results
- 100% customer driven – no government mandates or reporting to government.
- Some companies actually choose to use in-house laboratories if they are not required to use third party laboratories; there are pros and cons to this.

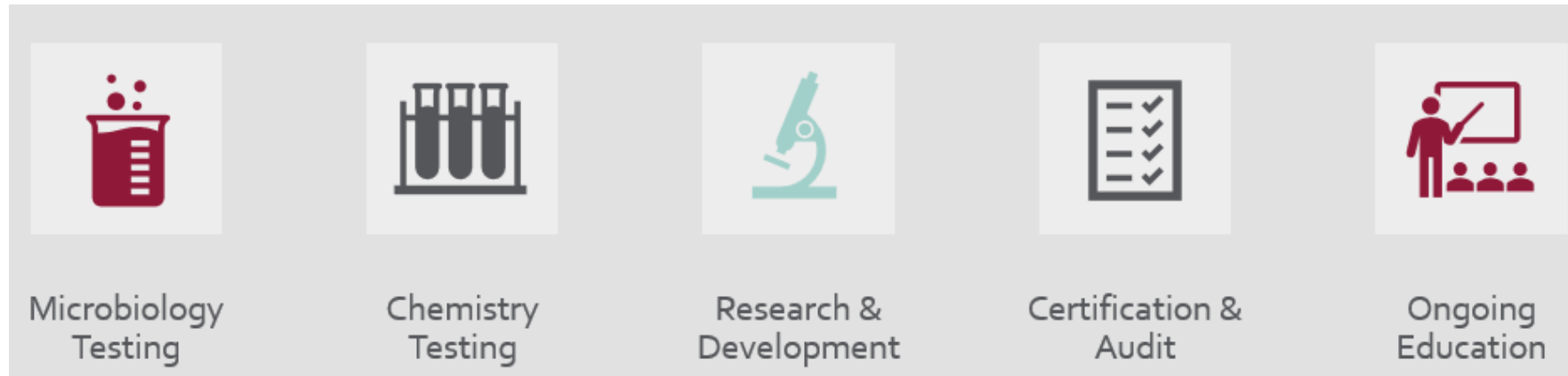
Basic Third-Party Laboratory Process Flow

1. Sample Receipt and Verification
2. LIMS Entry
3. Workflow Assignment
4. Sample Setting
5. Sample Incubation
6. Sample Testing / Enumeration
7. Result Recording
8. Data Entry
9. First and Second Approval
10. Result Release to Client



Certified Group Snapshot

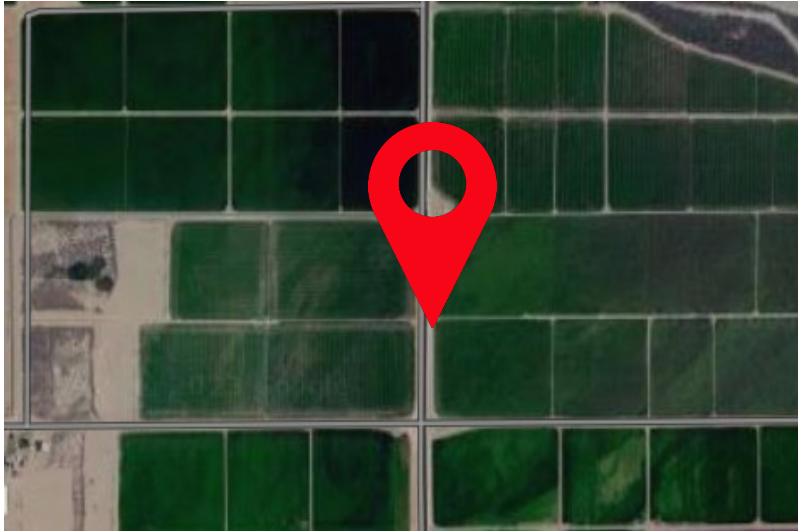
- Certified Group is a network of **state-of-the-art ISO-17025 accredited** third-party laboratories across North America, **open 24 hours a day, 365 days a year.**
- Certified Group conducted over **seven million tests** in 2022 at our 29 lab facilities, for over 4,000 customers.
- We provide **microbiological and chemical analyses** of all food matrices and environmental samples, as well as **education, consulting, and research & development.**
- Additional services include **BRC, SQF, FSSC, GFSI, and PAACO approved Certification & Audit** capabilities, and **Testing Supplies** through the SciCo Supply division.
- Since inception in 1926, our labs have been providing unsurpassed service to our food industry partners. The **96 years of experience and knowledge** of our network is unmatched by any other organization.



Sample Accessioning

Sample Delivery Logistics

- Timely delivery of results starts with a timely pickup of samples.
- The produce industry needs a fast turn-around-time, so on-time pickup of samples is critical.
- Overnight shipments typically will not work for the produce industry's short time frame between sampling and harvesting. Adds too many hours and will hold up ag operations.
- Thus, the laboratory needs to be located near the fields and needs to have a reliable courier team to ensure samples are in good condition at pickup and are delivered on time.



Laboratory Hours of Operation

- Laboratories must also be able to receive and accession the samples at the times that the courier drops them off so as not to delay sample processing.
- Most third-party laboratories operate 24/7/365 so that samples can be received when the courier drops off samples and then start sample processing right away.
- Again, we do not want to delay processing and want to release results as soon as they are ready so as not to hold up the grower's ag operations.



Accessioning of Samples

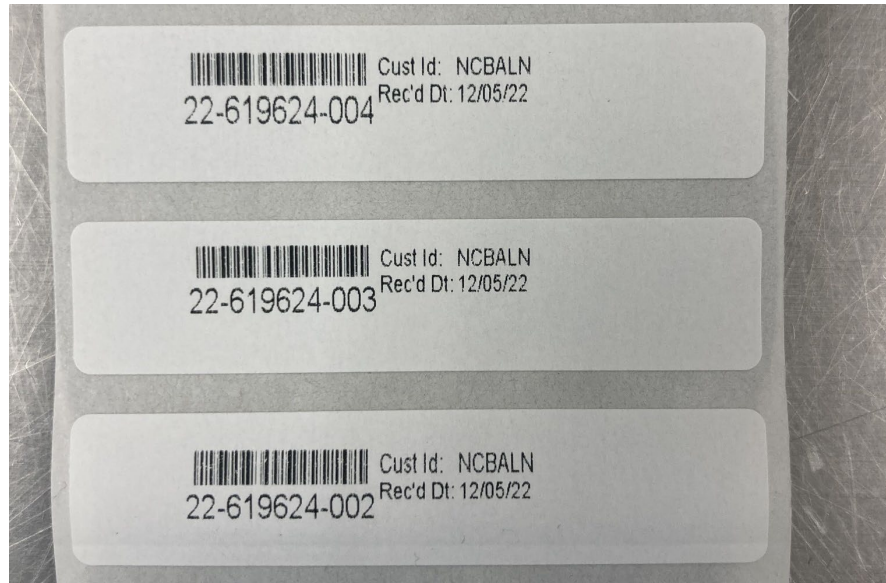
- As soon as the samples arrive at the laboratory, the first step is accessioning.
- Very important step! If an error is made at the beginning, it affects everything downstream!
- Login Department is dedicated to handling sample accession work.
- Open coolers, check temperature (< 10°C), and check contents for integrity.
- Check sample identifications on sample bags and pair up with sample submission paperwork or with electronic submission information.



FSNS [®] Sample Submission Form		Page ___ of ___	
<small>Submit to Food Safety Net Services (Check One Location):</small> o 258 W. Turbo Dr, San Antonio, TX 78216 / Ph. 210-308-0675 o 2545 114th St., Grand Prairie, TX 75050 / Ph. 972-602-2078 o 6215 W. VanBuren St., Phoenix, AZ 85043 / Ph. 602-385-4030 o 3400 S. Packerland Dr. De Pere, Ste 102, WI 54115 / Ph. 920-465-4165 o 1707 Stone Ridge Dr., Tucker, GA 30083 / Ph. 770-788-1940 o 186 S West Ave., Ste. 104 Fresno, CA 93706 / Ph. 559-443-1046 o 6281 Chiles Dr., Commeros, CA 90040 / Ph. 562-806-2143 o 4190 Fisher Rd., Columbus, OH 43228 / Ph. 614-274-2070 o 331 N. Nelson St., Amarillo, TX 79107 / Ph. 806-576-4065 o 3559 N Thompson St., Springdale, AR 72764 / Ph. 479-231-1250 o 351 N Mitchell St., Ste. 300 Boise, ID 83704 / Ph. 208-513-2020 o 5303 F St., Omaha, NE 68117 / Ph. 402-970-0280 o 8750 West 20th St., Greeley, CO 80634 / Ph. 970-372-3800 o Range Rd 145 A Newell County No. 4, Brooks, AB Canada / Ph. 888-525-9788 o 7020 Snowdrift Rd., Allentown, PA 18106/ Ph. 610-295-0210 o 410 N 200 W., Hyrum, UT 84319 / Ph. 888-525-9788 o 1519 S. 2nd Ave., Dodge City, KS / Ph. 620.682.7202			
Company Name:	Test Company	Contact Name:	Food Safety Manager
Address:	12345 Food Safety Way	City, State:	Sample, TX 71111
Email:	foodsafety@testcompany.com	Phone:	210-111-1111
Special Instructions/Notes:			
Project Information (optional):			
Project Name:	Ref / P.O. #:	Sampled By:	
Project #:			
<small>Laboratory analytical services performed by FSNS shall be governed by FSNS's terms and conditions, contained in the form Client Packet that can be viewed at https://www.foodsafety.net/locations/client_packet_form. By submitting samples for testing, Client agrees to be bound and to accept these terms and conditions. These terms and conditions shall control unless and until Client and FSNS have executed a Client Packet or a separate services agreement with different terms and conditions that control.</small>			
Sample #	Sample Date:	Identify Composite s (if any)	Sample Type (i.e. meat, supplement, water, sponge, poultry, etc.)
	Sample Description (List below as applicable)		
1	Sample A	None	Lettuce
2	Sample B	None	Lettuce
Check on row corresponding to the Sample Description for analysis to be performed			
Analysis		Salmonella	
Method		BAx	
Specifications		375 g	
Test Code		SAL100	
		X	
		X	
Courier/Receiver - Initial _____ Date _____ Time: _____ am/pm (Optional - Completed by Request)			
Control # FSNS-ADMIN-FORM-018.13 Revised On: February 11, 2021 Effective On: February 26, 2021 Authorized By: Corp. Quality Assistant Reviewed By: VP of Quality & Training			

Accessioning of Samples

- All samples are logged into LIMS (Laboratory Information Management System) and are assigned to proper workflows for testing.
- All samples are labeled with a unique sample identification that is generated from LIMS.
- A second check on workflows is done before the samples are released for setting.
- Samples are stored in a walk-in cooler (if needed) until they are set.



Sample Setting

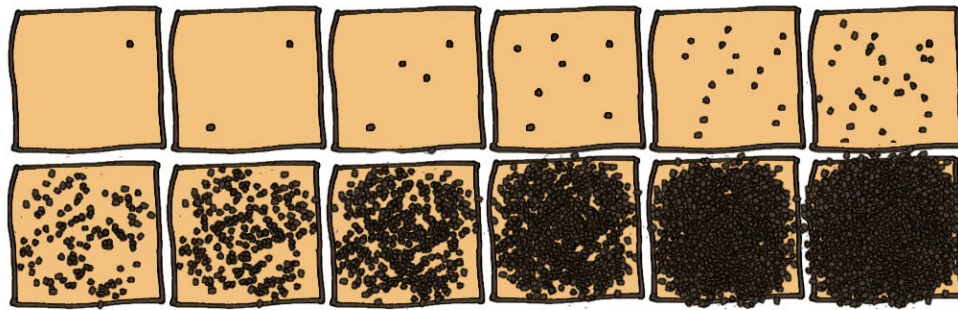
Setting Samples

- Once workflow assignments and reviews are done, the samples will be set in the lab.
- Needs to occur as soon as possible to prevent delays. Typically will not wait and batch.
- Main Lab Department is dedicated to handling sample setting.
- Critical to maintain high standards of cleanliness and sanitation.
- Do not want to cross-contaminate samples. Color coding lab coats/separating work areas.



Setting Samples

- Will verify the workflow, and will obtain the correct enrichment media to use.
- Enrichment media provides the water and nutrients that bacteria need to multiply so that one cell can become billions of cells that can be easily detected by testing assays.
- For methods with fast turn-around-times, pre-warmed ($\sim 42^{\circ}\text{C}$) enrichment media is usually required to minimize the time required for incubation. Bacteria need warm temperatures ($35\text{-}42^{\circ}\text{C}$) to start multiplying, so this gives the samples a head start. Do not need to wait for the sample incubator to warm up the media.



It doesn't take long for bacteria to go from one...to a million!



Setting Samples

- In some cases, concentrated enrichment media is used, so pre-warmed sterilized water from a water table is added to the samples along with pre-warmed concentrated media.
- The correct amount of enrichment media is delivered according to the amount of sample.
 - Typically a 375 g sample is combined with 1500 ml (1:5) or 3375 ml (1:10) of media.
 - Produce field 1500 g samples are typically divided up into 375 g samples. 1500 g is too large to enrich with standard laboratory bags – would require up to 6 Liters (1:5) or 13.5 Liters (1:10) of media!
 - 6 Liters = 1.5 gallons / 13.5 Liters = 3.5 gallons! We would need buckets to hold samples!



Setting Samples

- After adding enrichment media, the samples will be homogenized either mechanically with a stomacher or by hand massaging (hand massage is common for most produce samples).
- Homogenization ensures that bacteria are released into the enrichment media from the samples. Complex three-dimensional structures of the plant tissue make it necessary to mechanically disrupt the attachment of the bacteria to the plant tissue.



Incubation

- Proper incubation temperature and time is key. As mentioned, bacteria multiply only when they have ideal temperatures for growth (35-42°C), and they need sufficient time at those temperatures in order to achieve the numbers needed for detection.
- Thus, the samples need to be placed in a sizeable incubator that has uniform temperature (checked by temperature uniformity studies) that does not have significant fluctuations.
- Need to be stacked into bins so they do not fall over, but also need to have adequate air flow around them in order to maintain proper temperatures.



Sample Testing

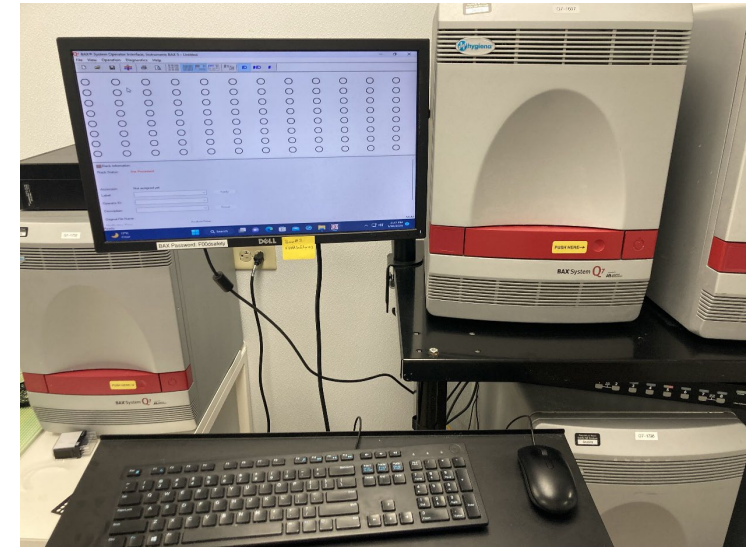
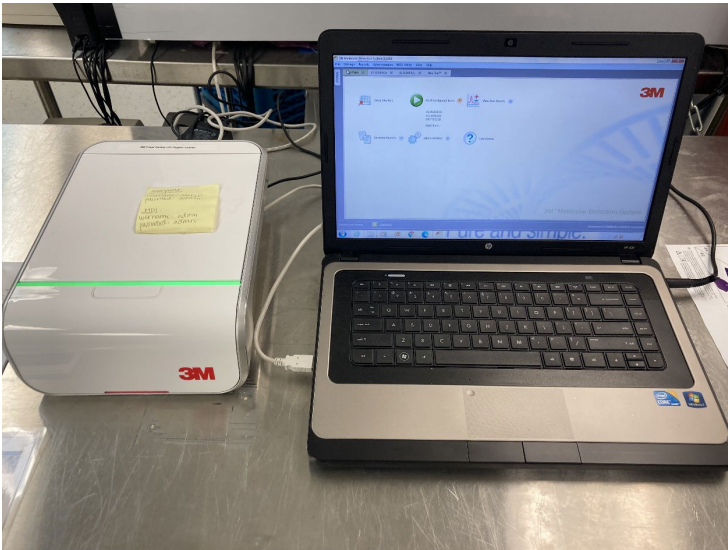
Testing Samples

- Downstream testing is done in the Pathogen Lab Department, which is staffed with personnel that are highly-trained on different testing technologies and methods.
- Must maintain high standards of cleanliness and sample separation, especially with regard to handling byproducts of DNA-based methods (amplicon).
- These personnel are also responsible for performing data entry and approval of reports.



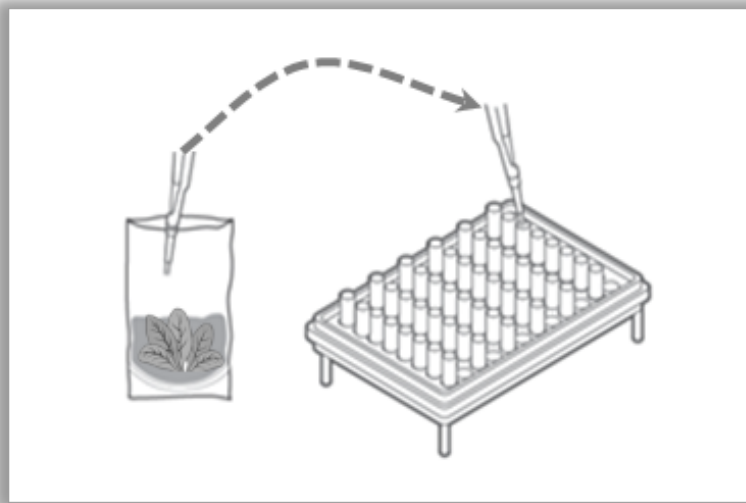
Testing Samples

- After incubation, the next step is to perform a detection assay.
- Incubation time will vary according to the detection assay that is being used. All incubation times and temperatures used must be validated for that detection assay.
- All detection assays used must have AOAC PTM or AOAC OMA validation certificates.
- If produce samples were not included on the AOAC PTM or AOAC OMA certificate, then an internal validation study should be done according to AOAC guidelines.

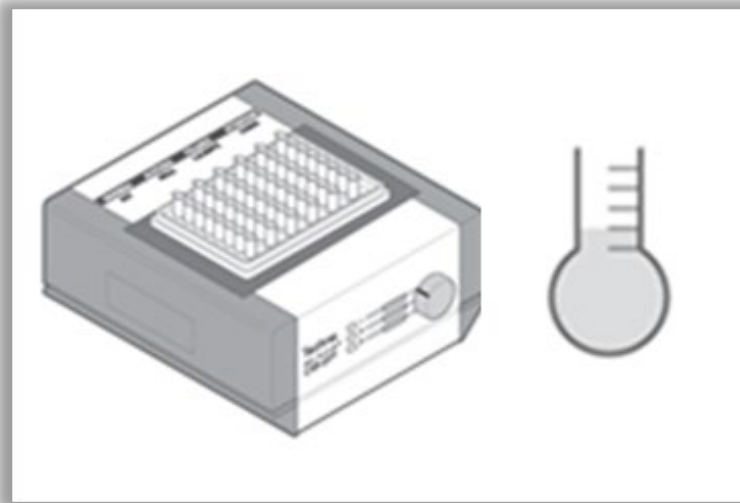


Preparatory Procedures

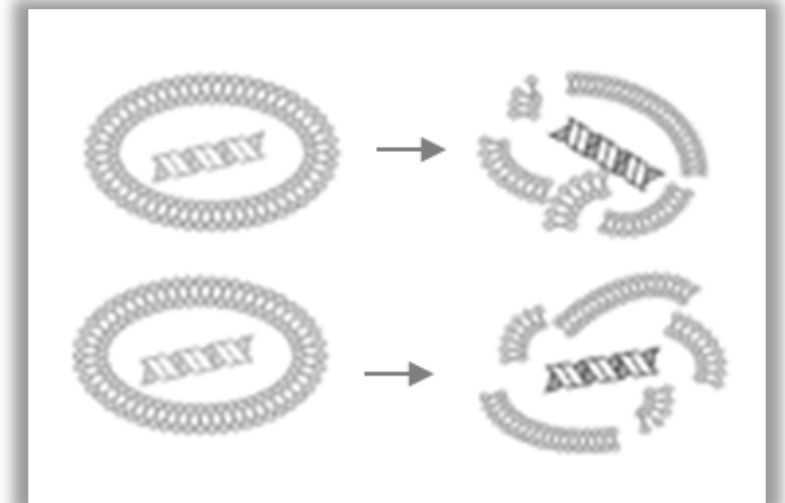
- Sample enrichments are typically subjected to a preparatory procedure.
- Preparatory procedures usually involve some sort of thermal, chemical, and/or mechanical lysis procedure in order to release the target DNA or antigens into a buffer.
- This allows the other reagents of the testing assay to have access to this target DNA or antigens in order to perform detection.



TRANSFER ENRICHMENTS



HEAT AND LYSE CELLS

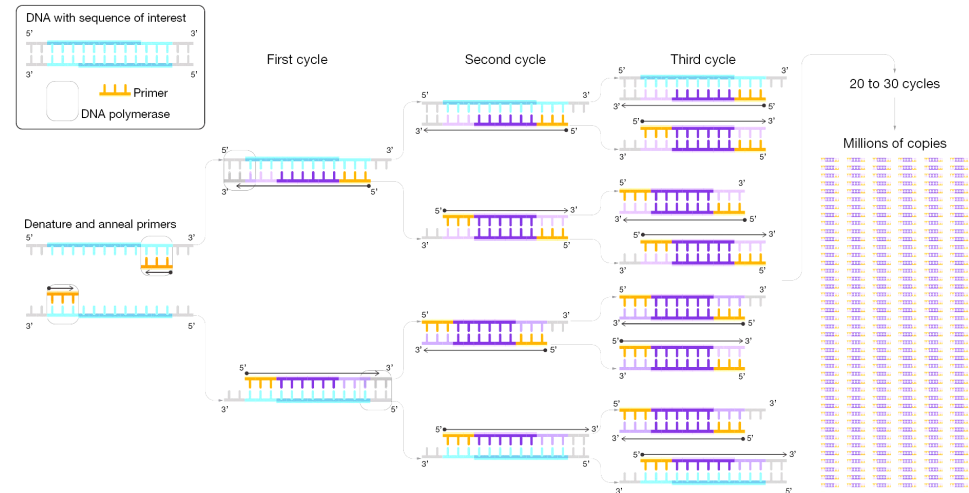
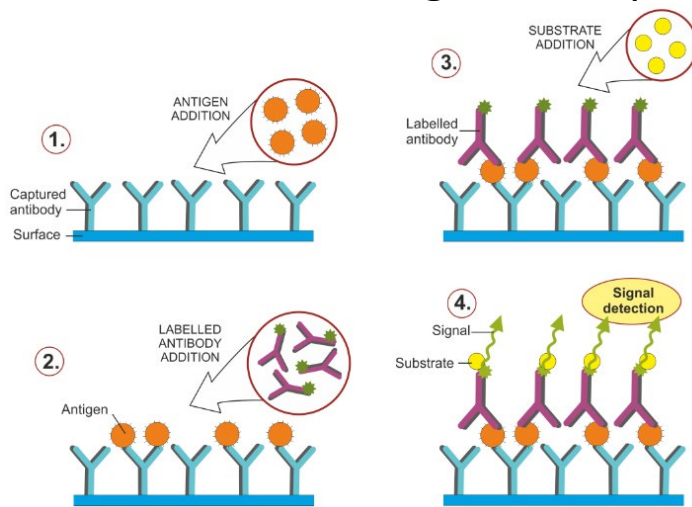


OBTAIN FREE DNA/ANTIGENS

Testing Samples

- In general, produce tissue testing is done for the pathogens *Salmonella enterica* and *Shiga Toxin Producing E. coli* (including *E. coli* O157:H7 and/or *stx1* or *stx2* and, minimally *eae* as the primary genetic attachment factor target) as per the LGMA guidelines.
- Different types of technologies can be used for detection:
 - **Antigen Binding:** involves pairing an antigen from the pathogen bacterial cells with an antibody or other receptor (such as a phage) that is part of the detection assay. Not able to be used for *stx/eae* testing.
 - **DNA Amplification:** involves amplifying a piece of DNA from the cell that is specific to the pathogen bacterial cell and detecting that amplification (can be fluorescent probes, bioluminescence, etc.).

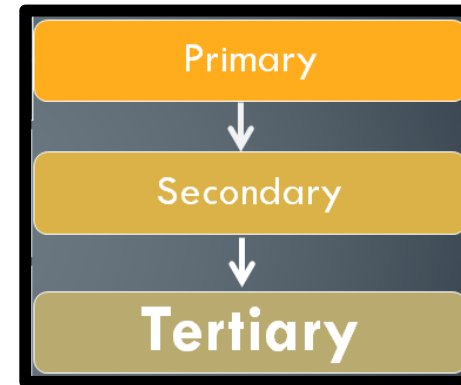
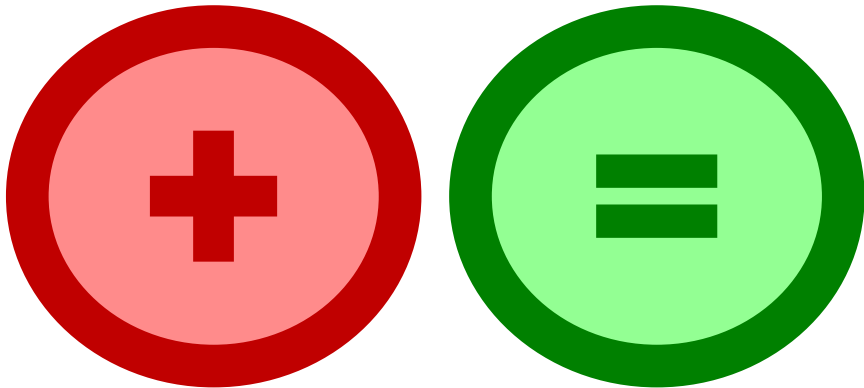
ANTIGEN BINDING



DNA AMPLIFICATION

Testing Results

- After detection assays are performed, results will be provided by the software.
- It is critical for the laboratory to be absolutely certain results are unequivocal.
- Any potential contamination issues need to be investigated (e.g. checking for green fluorescent protein-marked control strains).
- As a lab, we understand that a positive result will create a host of downstream actions for the customer. Thus, all results are thoroughly reviewed, but especially positive results.
- All results are entered into LIMS (preferably via electronic LIMS linking from the detection assay to LIMS to reduce errors), and undergo a tertiary review prior to release.



Testing Results

- Customer will receive results via a Certificate of Analysis via a LIMS email or via another customized data reporting system such as a direct LIMS to ERP link.
- Depending on specifications, a positive notification email/text/call may be sent.
- The customer can then decide to take the result as is, perform further detection testing (if applicable/validated), or perform a cultural confirmation.
- Cultural confirmations are performed according to the FDA Bacteriological Analytical Manual (BAM) and can add several more days of testing.



Analytical Results

Report Number: 23-048409
Report Date:

258 W. Turbo, San Antonio, TX, 78216 Phone:210-308-0675 Fax:210-308-8730

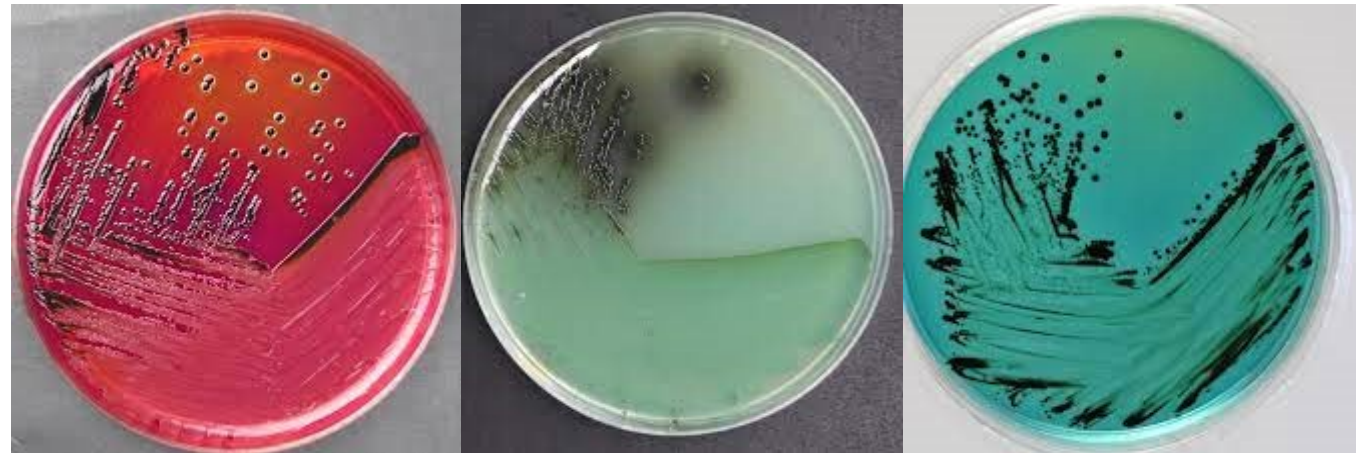
Customer*: Food Safety Net Services - San Antonio Special Projects

Contact*: Alex Brandt
258 W. Turbo

Samples Received: 01/27/2023
Start of Testing: 01/27/2023
PO Number:

San Antonio, TX, 78216
Phone*: 210-308-0675
Fax*: 210-384-3433

Billing Code	Sample Date*	Sample Number	Sample Description*	Analysis - FSNS Method Number	Result	Units
APC05	01/27/2023	001	Alex's Test Sample Composite: None	Aerobic Plate Count Petrifilm FSNS # 1.3 (AOAC)	10	CFU/





Factors To Consider When Evaluating Laboratories

Customer and Laboratory Relationship

- Relations between the customer and lab should be the primary factor for evaluation.
- Customer needs to be able to trust the third-party laboratory.
- Relationship must be more than just samples and certificates of analysis.
- Needs to be a true partnership where each one understands the other's needs.
- Need to be able to have open and clear communication at all times, especially when errors occur. Need to be able to discuss what happened and what is being done to correct it.



Laboratory Capabilities

- Another essential factor for choosing a third-party laboratory is its scope of capabilities.
- Some special tests require special equipment and not all laboratories are set up with that equipment, nor do they have the training in some particular methods.
- Third-party laboratories have an advantage over in-house laboratories in that they often have many more options for testing the same analyte. Thus, have backups in case of issues.



Accreditation

- ISO/IEC 17025 is a standard that laboratories are accredited to in order to demonstrate their technical competence.
- Specifies management and technical requirements for a laboratory to carry out tests or calibrations.
- Laboratories will have a scope of methods that are covered under their ISO/IEC 17025 accreditation.
- Third-party accreditation bodies (e.g. A2LA) will then perform assessments on third-party laboratories to demonstrate their compliance to the ISO/IEC 17025 standard.
- On-site audits, quality record reviews, and direct observation are performed by the third-party accreditation body.
- The third-party accreditation body is then also accredited by another certification body (e.g. ILAC)



Cleanliness and Organization



Other Important Factors to Consider

- Adherence to Good Laboratory Practices/Quality Management
- Location of Laboratory
- Hours of Operation
- Expertise in Produce Testing
- Technical Resources
- Ability to Scale Up and Handle High Volume Periods





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T H A N K Y O U

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