**PathogenDx Assay Descriptions**

PathogenDx offers a series of nucleic acid microarray diagnostic assays (Detect^X, Enviro^X, and Quant^X) for pathogen testing in food, agriculture, and environmental safety. Each of the PathogenDx diagnostic assays offer:

- Rapid detection and/or quantitation in 6-8 hours without sample pre-enrichment.
- High degree of species or indicator class specificity using unique probe sequences.
- High assay sensitivity (Detect^X - down to 1 CFU/gram) to meet the demands of state regulations.
- The ability to analyze raw sample lysate without the need for DNA purification or normalization.
- The ability to multiplex up to 44 unique organisms in triplicate.
- All in one low cost platform with multiple revenue generating opportunities.

While the microarray content and highlighted purpose may differ among the different assays. The same basic laboratory workflow and equipment used remains the same. As highlighted in the diagram below each assay will be performed using the same sample processing, sample lysis, two-step PCR reactions, slide hybridization and image scanning and analysis. The difference in each assay is related to the PCR primer mixes that are used, the PCR conditions for each assay, and the specific microarray content being analyzed. This makes it easy to implement multiple PathogenDx assays in the same lab, requiring minimal additional training and no additional equipment requirements.

DEFINITIVE, QUANTIFIED DNA-BASED TEST RESULTS IN 6 HOURS OR LESS.
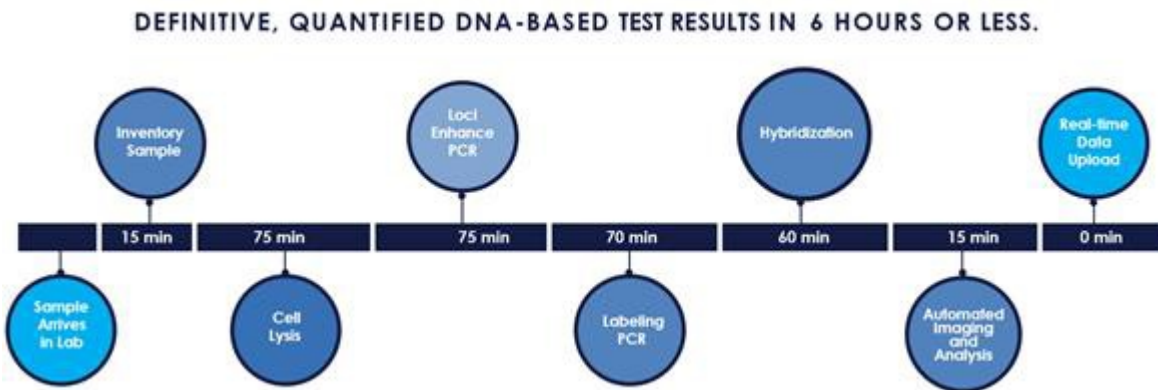
Assay Comparison	Detect^X	Enviro^X	Quant^X	Enviro^{X-F}
Qualitative or Quantitative	Qualitative	Qualitative	Quantitative	Qualitative
Diagnostic or Screening Tool	Diagnostic	Screening	Diagnostic	Screening
Species Specific Detection	Yes	Yes	No	Yes
Sample Enrichment	No	No	No	No
Single/Multiplex	Multiplex	Multiplex	Multiplex	Multiplex
Time to Result	>8 Hrs	>8 Hrs	>8 Hrs	>8 Hrs

Detect^X

Detect^X is a highly robust, sensitive and specific diagnostic assay designed to provide a presence or absence readout if the organism is present at or above 1 CFU/gram. This assay has the shortest processing time of any multiplex microbial assay accepted by a regulatory agency and is able to test a wide variety of matrices.

The Detect^X assay allows the user to detect *E. coli* species, both pathogenic and non-pathogenic, at levels down to 1 CFU/gram and allows the user to differentiate between Shigatoxigenic (Stx1 and Stx2) pathogenic strains. *Aspergillus* is a large and extremely diverse genus of fungi, with many species resembling one another. Detect^X will speciate four specific *Aspergillus* species (*A. flavus, A. fumigatus, A. niger,* and *A. terreus*), and reliably differentiate them from similar, but less pathogenic species of *Aspergillus*.

Bacterial Array Content	Fungal Array Content
Escherichia coli specific (< 1 CFU/gram)	*Aspergillus flavus* (< 1 CFU/gram)
Escherichia coli/Shigella spp. (< 1 CFU/gram)	*Aspergillus fumigatus* (< 1 CFU/gram)
Shigatoxin Stx1 gene (< 1 CFU/gram)	*Aspergillus niger* (< 1 CFU/gram)
Shigatoxin Stx2 gene (< 1 CFU/gram)	*Aspergillus terreus* (< 1 CFU/gram)
Salmonella specific (invA) (< 1 CFU/gram)	
Clostridium Botulinum (Florida and New York)	
Staphylococcus aureus (New York/ West Virgina/ Canada)	
Pseudomonas aeruginosa (West Virgina and Canada)	

Enviro[X]

Enviro[X] is a screening and monitoring diagnostic tool. The array contains probe sequences designed to detect a wide range of organisms known to be environmental contaminants or pathogens. The exclusion of a sample enrichment requirement ensures an accurate representation of the tested microbial population by avoiding sample enrichment bias. This assay is best applied as a monitoring, research, or screening tool. Possible uses may include investigating sources of contamination, testing the effectiveness of remediation methods, or screening potential facilities for pre-existing contamination.

Bacterial Array Content	Fungal/Yeast Array Content
Class Indicators	**Class Indicators**
Pan Bacterial (TAB)	Pan Fungal (TYM)
Bile-Tolerant Gram Negative (BTGN)	
Enterobacteriaceae (ENT)	
Species Indicators	**Species Indicators**
Salmonella/ Enterobacter	Aspergillus flavus
Salmonella spp	Aspergillus fumigatus
Escherichia/Shigella	Aspergillus niger
Escherichia stx1	Aspergillus spp
Escherichia stx2	Aspergillus terreus
Escherichia eae	Botrytis spp.
Pseudomonas spp	Penicillium spp.
Pseudomonas aeruginosa	Fusarium spp
Listeria spp	Fusarium solani
Campylobacter spp	Fusarium oxysporum
Xanthamonas	Mucor spp.
Aeromonas spp	Histoplasma
Aeromonas hydrophila and A. salmonicida	Monocillium
Bacillus spp	Tricoderma
Vibrio spp	Chaetomium
Staphylococcus spp	Stachybotrys spp.
Staphylococcus aureus	Alternaria spp.
Hafnia	Phoma/Eppicoccum
Klebsiella	Golovinomyces spp. (Powdery Mildew)

Serratia	Pan Powdery Mildew
Klebsiella	Golovinomyces
Chromobacterium spp	Blumeria
Bacillus spp	Erysiphe
Streptomyces spp	Podosphaera spp
Legionella	Oidiodendron
Alkanindiges	Rhodotorula
Citrobacter	Cladosporium spp.
Clostridium spp	Candida albicans
Yersinia	Candida spp
Panteoa	Candida albicans/tropicalis/dubliniensis
	Penicillium/Aspergillus spp.
	Saccharomyces spp.

Quant^X

Quant^X is a quantitative microbiological diagnostic assay that will enumerate different bacterial or fungal indicator classes. This array is designed to meet the needs of state and country regulations where quantitation of Total Aerobic Bacteria (TAB), Bile-Tolerant Gram Negative (BTGN), Total Enterobacteriaceae (ENT; including Total Coliform) and Total Yeast and Mold (TYM) is required. The assay will establish linearity and quantitation of signal through the use of synthetic DNA fragments included in PCR and hybridization. The synthetic DNA fragment is designed to be similar in length and sequence structure to the target sequence, thus ensuring similar amplification during PCR. The fragment has multiple nucleotide mismatches purposely designed into the sequence, to ensure no cross-reactivity between the target sequence, and the synthetic DNA fragment. The assay will provide results as CFU/gram values, with standard deviations below those reported for traditional plate cultures.

Bacterial Array Content	Fungal/Yeast Array Content
Total Aerobic Bacteria (TAB)	Total Yeast & Mold (TYM)
Bile-Tolerant Gram Negative Bacteria (BTGN)	
Total Enterobacteriaceae (ENT) [Includes Total Coliform]	

Enviro^{X-F}

The PathogenDx AOAC PTM Enviro^{X-F} assay consists of sample DNA amplified via a tandem Polymerase Chain Reaction (PCR) as a crude lysate which avoids purification. The Cy3 labeled PCR product is used without amplicon clean-up, quantitation, or normalization prior to hybridization. The Cy3 labeled tandem PCR product is diluted in hybridization buffer, which is then hybridized to the microarray. The hybridized and washed microarray is then imaged to yield a Cy3 hybridization pattern distributed among the probe spots. The PathogenDx software analysis tool, Augury©, automatically finds the hybridized spots in the image and then calculates the median Cy3 intensity of each hybridized spot.

Bacterial Array Content
Salmonella sp
Listeria spp
Listeria mono
E. coli*
Stx1*
Stx2*
EAE*
Camplobacter spp*

PERFORMANCE TESTED
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