# Hybridization Protection Assay

The third and final level of specificity: Ensures optimal assay accuracy and confidence in results.

Hybridization Protection Assay (HPA) is a detection methodology utilizing a chemiluminescent marker for detecting pathogens. This detection method is the third level of specificity inherent to Roka Detection Assays. This stage is critical to ensuring accurate results and eliminating the risk of false positives. A highly specific probe, labeled with an acridinium ester (AE) molecule, is introduced to the sample. If the target is present, the probe will bind, forming a protective double helix around the light-emitting AE molecule. If there is no target present, the probe will not bind. A Selection Reagent is added to the sample, which hydrolyzes unbound AE molecules so they cannot emit light. Next, detection reagents are added, and if any target is present the protected AE molecules will emit light, thus distinguishing positive and negative samples.

# Benefits of Hybridization Protection Assay

- Clear positive and negative results
- High level of specificity with target-specific probe
- Chemiluminescent detection is not affected by colors or dyes intrinsic to specific food matrices
- Objective results remove subjective interpretation
- Reduced risk of false positives



## Probe and Target Amplicon Are Introduced



### **Description:**

- A highly specific probe, complementary to a sequence of the pathogen, is introduced to the sample reaction
- The probe is labeled with a single acridinium ester molecule

## Probe Binds to Target Amplicon



Probe Binds to Target

#### **Description:**

- The probe binds to the target nucleic acid sequence
- The acridinium ester molecule is protected by the double helix formed by the target amplicon and probe
- Any unbound probes are cleaved after adding the Selection Reagent

# Light Emission



#### **Description:**

- The probe/target amplicon complex is moved to the luminometer
- The Auto Detect Reagents are injected into the sample tube
- Upon addition of Auto Detect Reagents, acridinium ester protected by the double helix will emit light. This "light" is read and interpreted by the Atlas<sup>®</sup> System which provides a positive or negative result.



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