

Microbial identification by immunohybridization assay of artificial RNA labels.

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Ribosomal RNA (rRNA) and engineered stable artificial RNAs (aRNAs) are frequently used to monitor bacteria in complex ecosystems. In this work, we describe a solid-phase immunocapture hybridization assay that can be used with low molecular weight RNA targets. A biotinylated DNA probe is efficiently hybridized in solution with the target RNA, and the DNA-RNA hybrids are captured on streptavidin-coated plates and quantified using a DNA-RNA heteroduplex-specific antibody conjugated to alkaline phosphatase. The assay was shown to be specific for both 5S rRNA and low molecular weight (LMW) artificial RNAs and highly sensitive, allowing detection of as little as 5.2 ng (0.15 pmol) in the case of 5S rRNA. Target RNAs were readily detected even in the presence of excess nontarget RNA. Detection using DNA probes as small as 17 bases targeting a repetitive artificial RNA sequence in an engineered RNA was more efficient than the detection of a unique sequence.

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