A Novel RNA *In Situ* Hybridization Method for the Detection of Mycobacteria in Clinical FFPE Samples

Na Li, Zhifu Zhang, Li-chong Wang, Wei Wei, Courtney Anderson, Robert Monroe, Xiao-Jun Ma
Advanced Cell Diagnostics, 7707 Gateway Blvd, Newark, CA 94560 USA

USCAP 108TH ANNUAL MEETING UNLOCKING YOUR INGENUITY
MARCH 16-21, 2019
Gaylord National Resort & Convention Center
National Harbor, Maryland
USCAP

Ab#: 1927

Background

Acid fast staining (AFS) for the detection of mycobacteria and other acid-fast organisms provides a rapid way of identifying pathogenic organisms in FFPE tissue in contrast to culture methods, which require weeks to perform. While rapid and straightforward, there are several issues with AFS. First, AFS can be challenging and time-consuming to interpret. Second, standard tissue fixatives and processing alters the lipid-rich cell wall of acid-fast organisms which reduces the detection sensitivity in FFPE samples. Last, AFS does not allow for discrimination between various acid-fast organisms, such as mycobacteria tuberculosis (MTB) vs. non-tuberculous mycobacteria (NTM). To address these issues with AFS, we have developed a highly sensitive and specific RNA *in situ* hybridization (RISH) assay using the RNAscope technology for the detection of mycobacteria rRNA in FFPE tissues and distinction of MTB and NTM.

Design

Serial sections from 50 FFPE lung samples from patients with a clinical diagnosis of pulmonary TB (not histologic) were collected from five hospitals in China. Samples were tested with AFS or using the RNAscope probes B-MTB-23s-rRNA-1-C1 and B-MTB-NTM-16srRNA-O1 targeting MTB only or both MTB and NTM, respectively, using a modified RNAscope protocol (Protocol I). A subset of the RISH positive samples were subsequently examined using an alternative protocol (Protocol II), which eliminates one pretreatment step (Figure 1). AFS and RISH slides were then analyzed for the presence of positively stained organisms.

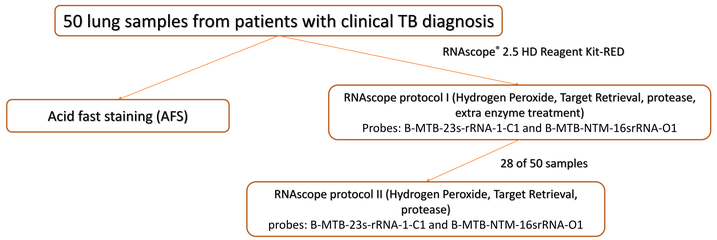
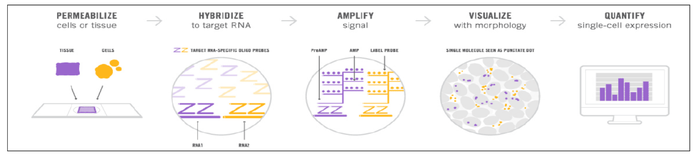


Figure 1. RNAscope ISH assay overview

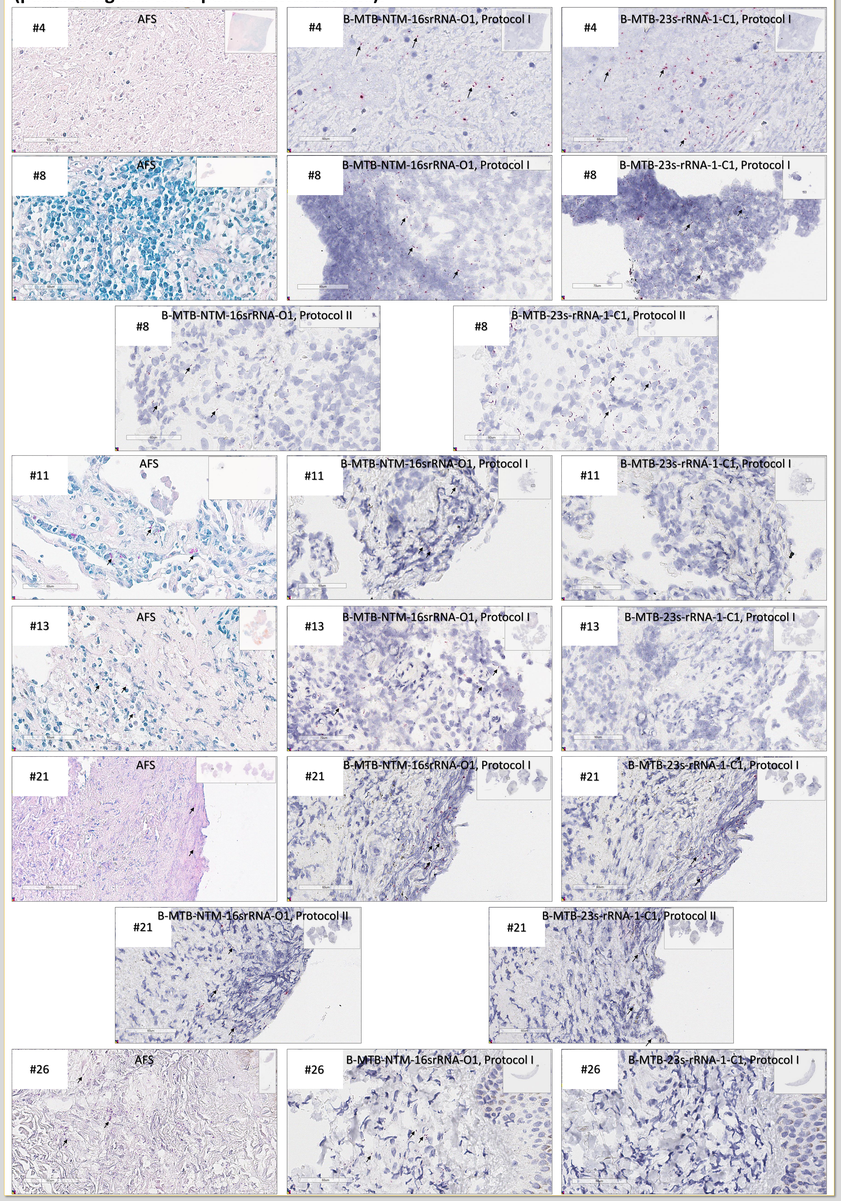


Results

Of the 50 TB patient samples, 36 showed AFS-positive organisms while 14 were negative. 38/50 exhibited positive RISH signals with the MTB-NTM probe, whereas 35/50 exhibited positive RISH signals with the MTB-specific probe. Of the 3 MTB-NTM$^+$/MTB$^-$ cases, all 3 were AFS-positive. 28 MTB-NTM$^+$ samples tested using Protocol II yielded the same results (Figure 2 and Table 1).

Results

Figure 2. Representative images of TB samples detected by AFS and by RNAscope ISH methods (positive signals were pointed with arrows).



Results

Table 1. Summary of 50 lung samples from patients with clinical TB diagnosis tested with AFS and RNAscope ISH assays. N/A, not applicable.

Sample number	Acid fast staining (AFS)	RNAscope 2.5 Reagent Kit-RED, Protocol I	RNAscope 2.5 Reagent Kit-RED, Protocol I	RNAscope 2.5 Reagent Kit-RED, Protocol II	RNAscope 2.5 Reagent Kit-RED, Protocol II
		B-MTB-NTM-16srRNA-O1	B-MTB-23s-rRNA-1-C1	B-MTB-NTM-16srRNA-O1	B-MTB-23s-rRNA-1-C1
1	-	-	-	N/A	N/A
2	-	-	-	N/A	N/A
3	-	-	-	N/A	N/A
4	**-**	**+**	**+**	N/A	N/A
5	-	-	-	N/A	N/A
6	+	+	+	+	+
7	+	+	+	+	+
8	**-**	**+**	**+**	**+**	**+**
9	-	-	-	N/A	N/A
10	+	+	+	+	+
11	**+**	**+**	**-**	N/A	N/A
12	+	+	+	N/A	N/A
13	**+**	**+**	**-**	N/A	N/A
14	-	-	-	N/A	N/A
15	-	-	-	N/A	N/A
16	-	-	-	N/A	N/A
17	-	-	-	N/A	N/A
18	-	-	-	N/A	N/A
19	-	-	-	N/A	N/A
20	+	+	+	+	+
21	**+**	**+**	**+**	**+**	**+**
22	+	+	+	+	+
23	+	+	+	+	+
24	+	+	+	+	+
25	+	+	+	+	+
26	**+**	**+**	**-**	N/A	N/A
27	+	+	+	+	+
28	+	+	+	+	+
29	+	+	+	+	+
30	+	+	+	+	+
31	+	+	+	+	+
32	+	+	+	+	+
33	+	+	+	+	+
34	+	+	+	+	+
35	+	+	+	+	+
36	+	+	+	+	+
37	+	+	+	+	+
38	+	+	+	+	+
39	-	-	-	N/A	N/A
40	+	+	+	+	+
41	+	+	+	+	+
42	+	+	+	+	+
43	+	+	+	+	+
44	+	+	+	+	+
45	+	+	+	N/A	N/A
46	+	+	+	N/A	N/A
47	+	+	+	N/A	N/A
48	+	+	+	N/A	N/A
49	+	+	+	N/A	N/A
50	+	+	+	+	+

Conclusions

In the current study we have developed a novel RISH method for the detection of mycobacteria in FFPE tissue. In comparison to AFS, the RISH methodology appears to be more sensitive, detecting 2 additional positive cases. In addition, the RISH method was able to identify 3 cases of probable NTM, a distinction of high clinical relevance owing to the different treatments required for MTB vs. NTM infections. Finally, the RISH signals were easier to visualize and locate compared to AFS. Overall, our results indicate that the RNAscope assay is a sensitive method to detect mycobacteria in FFPE tissues that allows for differentiation of MTB vs. NTM and is easy to interpret.