Use of a disposable lysis device and capture of unamplified mycobacterial rRNA on a liquid bead array for species determination from liquid cultures

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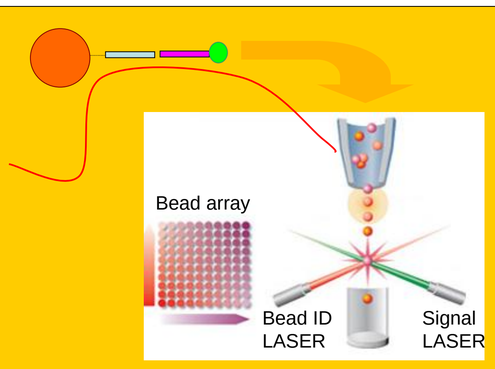
Background: Simple and rapid species identification from mycobacterial culture is critical especially when liquid culture is used.

The recent availability of antibody based lateral flow tests to confirm the presence of a member of the *M. tuberculosis* complex (MTBC) is a valuable advance but these strips do not currently allow other mycobacterial species to be identified. A method with the potential to allow a range of non-tuberculous mycobacteria (NTMs) to be simply and rapidly identified in addition to *M. tuberculosis* is appealing.



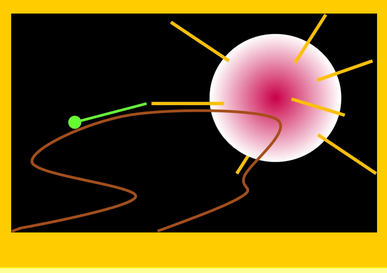
Here we explore the possibility of directly capturing and detecting unamplified rRNA from lysed cells using a liquid array system (Luminex corporation). The liquid bead array is composed of a series of magnetic micro-beads internally labeled with a ratio of two dyes which allows them to be identified in a dedicated reader (flowcytometer or CCD camera flow cell). In our proposed assay the beads are covalently coupled to oligonucleotides designed to capture specific mycobacterial rRNA molecules. This method has the advantage of avoiding an enzymatic amplification/labeling step wich is generally required for hybridization assays providing the potential to develop a robust and rapid method.

Direct capture and detection of bacterial ribosomal RNA (rRNA). The concept:

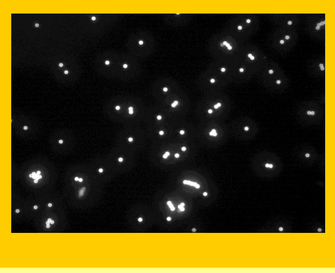
There are 1000 to 10,000 copies of rRNA present in an actively growing bacterial cell.

Our proposed assay relies on a series of identifiable micro-beads covalently coupled to oligonucleotides that specifically bind to mycobacterial rRNA. A second oligonucleotide containing a biotin label is also present and can hybridize to the targeted bacterial rRNA directly adjacent to the immobilized oligonucleotide.

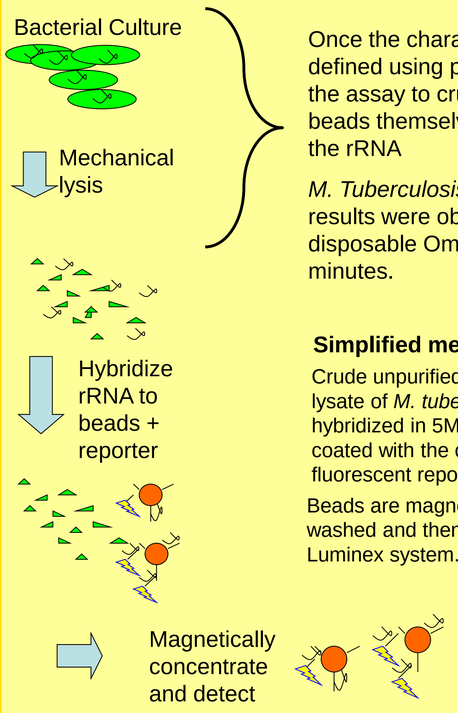
In this way, when the cell lysate contains bacterial ribosomes, the rRNA acts as a bridge between the immobilized and the labeled oligonucleotides and concentrate the signal at the bead surface where it can be detected. After washing of the (magnetic) beads the amount of label on each species of beads can be accurately determined by the Luminex device.



Cartoon of the proposed method (left – 6 µM bead red, immobilized probe yellow, fluorescent probe green, captured rRNA brown)

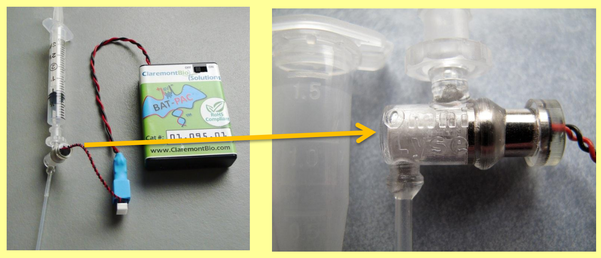


Fluorescent microscopic image of the beads (right).



Once the characteristics of the assay had been defined using purified whole cell NA, we applied the assay to crude bacterial lysate, using the beads themselves to capture and concentrate the rRNA

M. Tuberculosis cells are hard to lyse, but good results were obtained by lysing the cells with disposable OmniLyse devices within 2.5 minutes.



Simplified method:

Crude unpurified mechanical (zirconium) lysate of *M. tuberculosis* is directly hybridized in 5M GTC (overnight) to beads coated with the capture probe and the fluorescent reporter oligonucleotide.

Beads are magnetically concentrated, washed and then analyzed by the Luminex system.

Summary of the results of 100 MGIT Cultures tested in the RIVM (data shown on the right)

	Total	PosCont	Accurate ID	Expected Result	Incorrect
NTM	63	59	25	44	2
MTBC	36	36	34	34	1
Total	99*	95	59	78	3

* = one mixed culture excluded from the analysis

Proof of concept in the Luminex system:

In close collaboration with the RIVM, we assessed the system using 8 species of beads to identify the bacterial species and one to detect mycobacterial rRNA as positive control. Samples were both from patient materials and laboratory strains.

		Total signal				% Total signal					
RIVM	rCapA		Pos cont (	Abs (26)	Avi (27)	Gor (28)	Kan (34)	Malm (39)	Mar (44)	Tub (54)	Xen (65)
M abs	M abs	2789	32	20	7	6	6	8	6	6	8
M abs	Myc	8782	44	8	7	8	6	7	7	6	8
M abs	M abs	14574	44	29	4	5	3	3	4	4	3
M abs	M abs	13693	41	32	4	4	3	4	4	4	4
M abs	M abs	16281	41	33	3	4	3	4	3	4	4
M avi	M avi	8831	35	2	47	2	2	3	2	3	3
M avi	M avi	1688	24	8	21	8	8	8	6	10	8
M avi	M avi	6855	37	2	49	2	2	1	2	2	3
M avi	M avi	4220	27	4	42	5	5	4	4	5	4
M avi	M avi	6861	37	2	47	2	2	2	2	3	3
M avi	M avi	9017	31	4	44	3	4	3	3	4	4
M avi	M avi	8867	32	3	44	3	3	4	3	4	4
M avi	M avi	2255	27	5	35	5	6	5	4	6	6
M avi	M avi	3017	26	7	32	7	5	6	5	6	7
M chel	M abs	9153	40	36	4	3	3	3	3	3	4
M chel	M abs	1290	31	19	7	8	8	7	6	8	7
M chel	No result	879	12	12	11	11	9	11	10	11	13
M fort	Myc	3215	51	6	5	5	6	7	6	7	7
M fort	Myc	11287	65	4	4	4	4	5	5	5	4
M fort	Myc	5416	71	4	3	4	3	4	3	4	4
M fort	Myc	10595	66	4	4	5	4	4	4	5	5
M fort	Myc	11716	75	4	3	3	3	3	2	3	3
M gor	Myc	3574	56	4	4	11	4	4	8	4	4
M gor	Myc	6406	54	4	4	9	4	6	9	6	4
M gor	Myc	6734	62	2	2	10	2	2	14	2	3
M int	Myc	1973	47	6	7	6	7	7	6	6	8
M int	Myc	4277	53	4	6	6	12	6	4	5	5
M int	Myc	1962	28	8	9	7	12	11	8	8	9
M int	Myc	3679	67	3	4	4	6	4	4	4	4
M int	Myc	5180	45	5	6	5	12	7	5	7	7
M int	Myc	5432	40	6	8	7	13	7	5	8	6
M int	Myc	3543	48	6	7	6	8	7	5	6	6
M int mix	M kan	7551	45	2	3	2	30	8	3	3	3
M kan	M kan	1362	17	8	8	7	21	11	12	8	8
M kan	M kan	8148	36	2	2	2	35	15	2	2	5
M kan	M kan	11083	29	2	3	3	34	16	2	4	6
M kan	M kan	10414	29	3	3	3	38	14	2	4	6
M kan	M kan	8562	34	2	2	2	37	13	2	3	4
M kan	M kan	5188	33	3	3	3	35	9	3	4	5
M kan 1	M avi	3084	31	5	33	5	5	5	4	5	6
M kan 1	M avi	9970	40	3	40	3	3	2	3	3	3
M kan l	No result	833	11	11	9	11	11	11	12	10	14
M malm	Myc	5082	58	5	6	6	5	6	5	5	5
M malm	Myc	2495	53	6	5	6	5	8	5	5	6
M mar	No result	931	12	11	10	11	11	10	14	11	11
M mar	No result	858	14	10	11	9	11	12	10	12	11
M mar	M mar	3682	35	5	5	7	5	5	22	13	4
M mar	M mar	1752	23	8	9	8	6	6	23	9	6
M mar	M mar	9358	36	1	1	5	2	2	33	18	2
M muc	Myc	4008	63	3	4	5	5	5	5	5	5
M muc	Myc	1148	31	9	8	8	8	8	8	9	10
M peregri	Myc	5582	67	4	5	4	3	4	4	4	5
M peregri	Myc	7917	67	4	4	4	4	5	4	4	5
M scr	M kan	2185	17	6	5	6	34	12	7	6	8
M scr	M kan	10513	34	2	2	2	37	13	2	2	5
M scr	M kan	6560	29	2	2	2	40	16	2	3	6
M scr	M kan	1919	26	6	6	6	24	10	6	6	9
M simiae	Mtb	6482	47	4	3	5	5	3	5	24	4
M simiae	M kan	5407	32	3	3	2	37	13	3	3	5
M terrae	Myc	5749	71	3	2	3	2	3	5	9	3
M terrae	Mtb	5731	33	2	2	7	2	2	19	33	2
M terrae	M kan	9750	33	1	1	1	39	16	1	2	5
M xen	M xen	11633	33	1	1	1	1	1	6	1	54
M xen	M xen	9639	30	1	1	1	3	1	5	1	55
M xen	M xen	7777	31	1	2	1	1	1	6	1	54
Mtb	Mtb	11586	53	2	2	4	3	2	5	27	2
Mtb	Mtb	10475	49	2	3	4	3	2	4	30	3
Mtb	Mtb	9008	52	2	2	5	3	2	5	26	3
Mtb	Mtb	8336	53	2	2	5	3	3	5	25	2
Mtb	Mtb	5881	47	3	3	5	4	3	5	26	3
Mtb	Mtb	9865	54	2	2	4	3	2	5	26	3
Mtb	Mtb	6148	13	2	3	9	6	3	19	43	3
Mtb	Mtb	22672	24	1	1	11	7	1	22	32	1
Mtb	Mtb	16854	21	1	1	11	7	1	21	35	2
Mtb	Mtb	18800	19	1	2	12	8	1	21	34	2
Mtb	Mtb	19447	21	1	1	11	8	1	22	34	1
Mtb	Mtb	16071	22	1	1	11	7	1	21	35	1
Mtb	Mtb	4916	11	3	3	10	6	3	12	49	3
Mtb	Mtb	23751	15	1	2	12	8	2	19	40	2
Mtb	Mtb	13582	14	1	1	12	5	1	14	49	1
Mtb	Mtb	23691	16	1	2	12	7	2	19	40	1
Mtb	Mtb	9518	17	1	2	8	5	1	26	37	2
Mtb	Mtb	6612	15	2	2	8	5	2	26	39	2
Mtb	Mtb	7994	14	2	2	8	7	2	26	38	2
Mtb	Mtb	5114	12	2	3	8	5	2	26	39	3
Mtb	Mtb	2363	12	3	4	10	5	4	24	34	4
Mtb	Mtb	3398	10	3	3	8	6	3	25	38	4
Mtb	Mtb	8531	17	1	1	8	5	1	26	38	1
Mtb	Mtb	9498	15	1	1	9	6	1	27	37	1
Mtb	Mtb	7477	15	2	2	9	4	2	28	36	2
Mtb	Mtb	23360	17	1	1	11	7	1	28	34	1
Mtb	Mtb	2502	14	5	5	9	6	4	18	31	6
Mtb	Mtb	14198	16	1	1	9	6	1	30	35	1
Mtb	Mtb	19933	16	1	1	12	7	1	27	33	1
Mtb	Mtb	2551	13	5	4	7	6	5	21	32	6
Mtb	Mtb	22543	16	1	1	12	8	1	27	33	1
Mtb	Mtb	33189	18	0	1	13	8	1	27	31	1
Mtb	Mtb	12269	15	1	1	9	5	1	30	36	1
Mtb	Mtb	4157	17	3	3	8	3	3	22	37	3
Mtb BCG	Mtb	12532	15	1	2	11	5	1	19	45	1
Mtb BCG	Myc	2880	47	5	5	13	4	5	12	5	5

Conclusion: Our results show that unamplified, unlabeled rRNA can be captured, detected, and identified using a liquid bead array. Results are encouraging, but can be improved. Due to the genetic similarity of the targeted region the current test cannot differentiate between *M. kansasii* and *M. scrofulaceum* and maybe *M. simiae*, nor can it differentiate *M. abscessus* from *M. chelonae*.
One *M. kansasii* laboratory strain was identified by the rCapA as *M avium*. Here there is also no similarity among the 2 regions. One patient sample, identified by the RIVM as *M simiae* and assayed twice by the rCapA assay from 2 different MGIT cultures, was once identified as *M tuberculosis* by the rCapA assay and once as *M kansasii* like. Because of similarity in the 16S region targeted it is likely that the rCapA assay will not always separate *M kansasii* from *M simiae*. Two *M. terrae* laboratory strains were tested using the rCapA assay one was identified as *M tuberculosis* and one as *M kansasii*. This result is difficult to explain, because both the *M tuberculosis* and the *M kansasii* probe have very little reported similarity with the *M terrae* 16S region. Only one BCG out of 36 MTB complex tested was not identified by the rCapA assay.
Further work on this approach will, in addition to optimizing the protocols, include: extending the range of species identified by adding beads, exploring the use of more stable/brighter labels (quantum dots etc.) and alternative detection strategies (in collaboration with our MicroNed partners and others).

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