Electronic Supplementary Material (ESI) for Lab on a Chip.

SUPPORTING INFORMATION

A large-volume sputum dry storage and transportation device for molecular and culture-based diagnosis of tuberculosis

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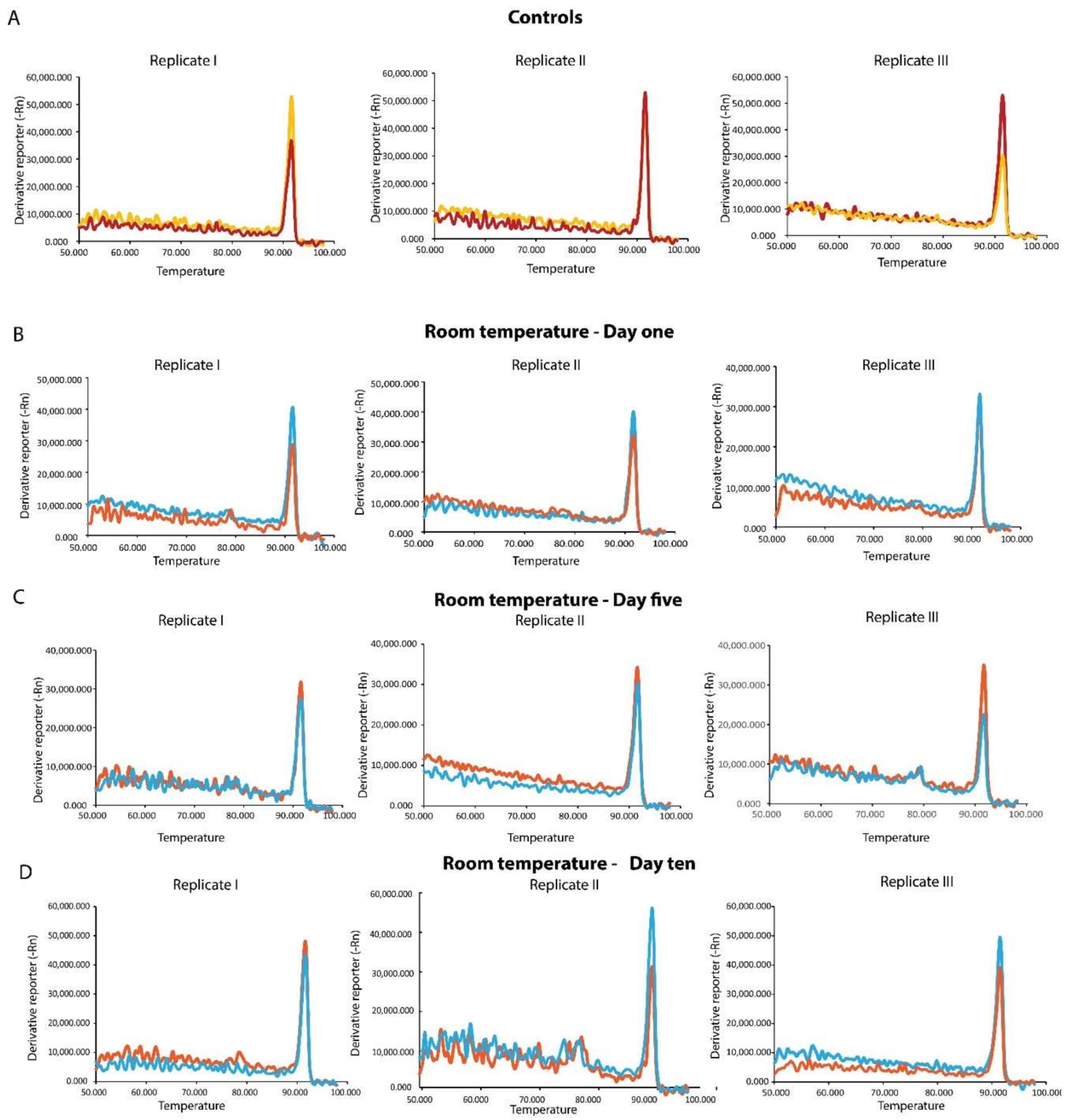
Supplementary Figure S1. Melt curve analysis of PCR amplicons for accelerated ageing experiments

All melt curves show single peaks, confirming that non-specific PCR products were not formed.



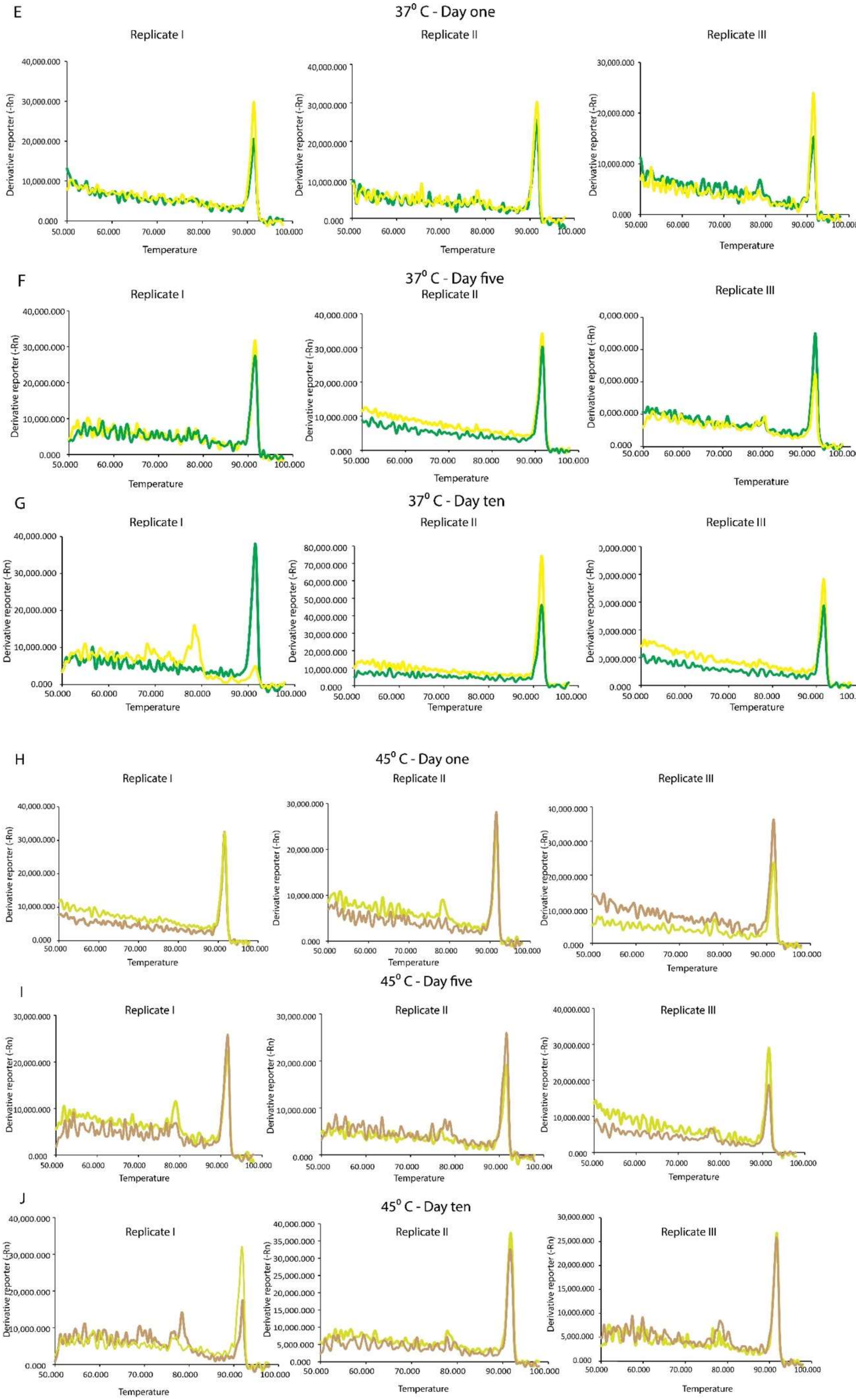

E
37⁰ C - Day one
Replicate I
Replicate II
Replicate III
F
37⁰ C - Day five
Replicate I
Replicate II
Replicate III
G
37⁰ C - Day ten
Replicate I
Replicate II
Replicate III
H
45⁰ C - Day one
Replicate I
Replicate II
Replicate III
I
45⁰ C - Day five
Replicate I
Replicate II
Replicate III
J
45⁰ C - Day ten
Replicate I
Replicate II
Replicate III
Derivative reporter (-Rn)
Temperature
50.000
60.000
70.000
80.000
90.000
100.000
0.000
10,000.000
20,000.000
30,000.000
40,000.000
50,000.000
60,000.000
70,000.000
80,000.000
5,000.000
15,000.000
25,000.000
35,000.000

Figure S1. Melt curve analysis of PCR amplicons for accelerated ageing experiments A. Controls. Fresh *Msm*-spiked sputum specimens that were not dry-stored. Red, yellow represents reaction duplicates in melt curve analysis. **B,C,D. Room temperature samples.** *Msm*-spiked sputum specimens dry-stored at room temperature and recovered on days 1, 3, and 5. Blue, orange represents reaction duplicates in melt curve analysis. **E,F,G. 37^{o}C samples.** *Msm*-spiked sputum specimens dry-stored at 37^{o}C and recovered on days 1, 3, and 5. Yellow, green represents reaction duplicates in melt curve analysis. **H,I,J. 45^{o}C samples.** *Msm*-spiked sputum specimens dry-stored at 45^{o}C and recovered on days 1, 3, and 5 respectively. Yellow, brown represents reaction duplicates in melt curve analysis.

Supplementary Figure S2. Gel electrophoresis analysis of PCR amplicons for accelerated ageing experiments

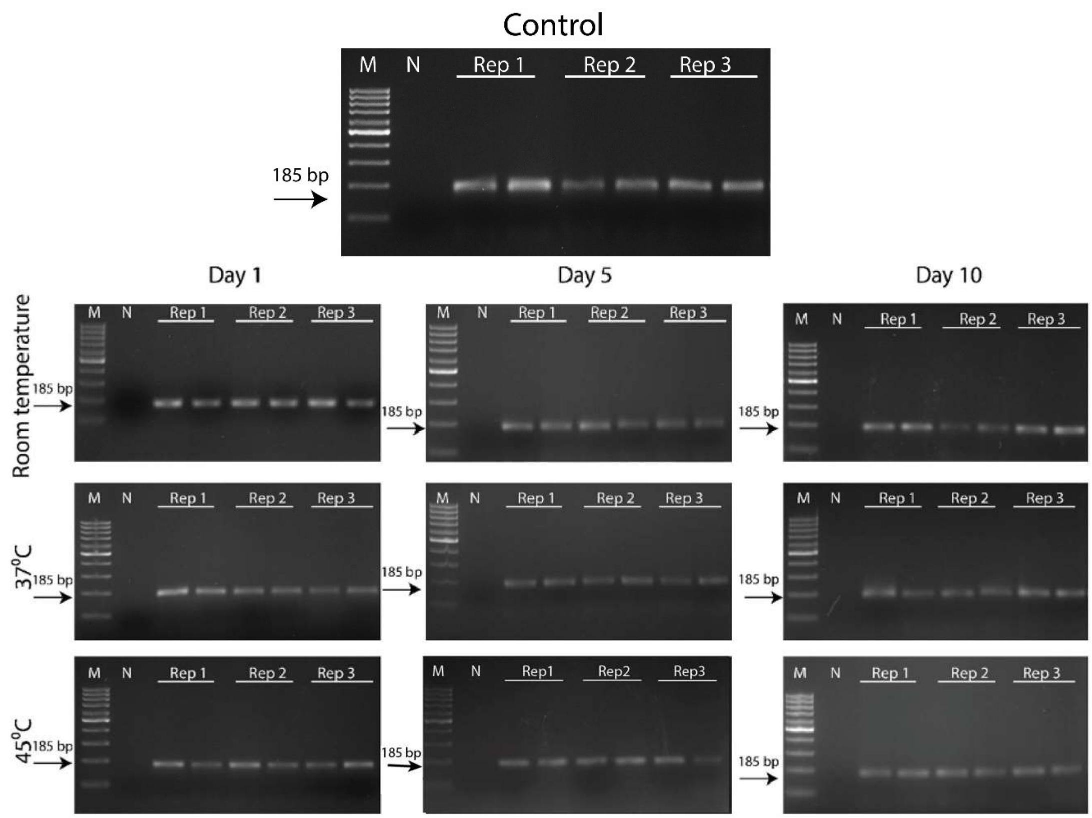
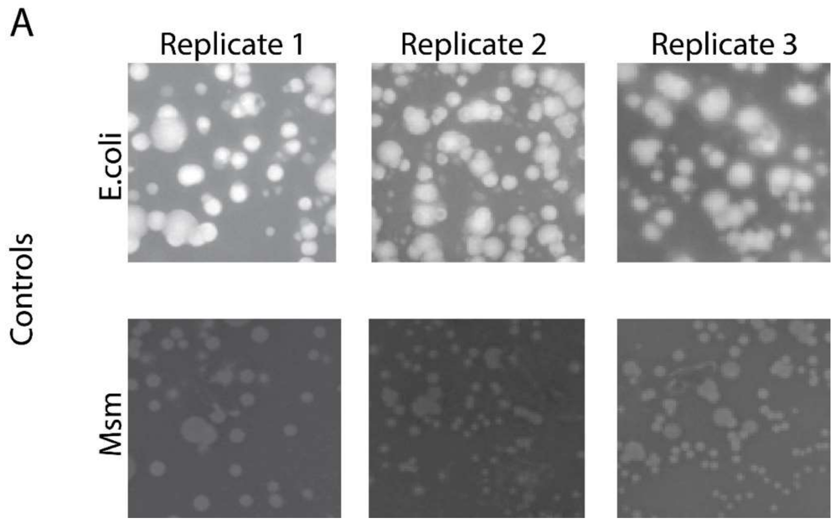
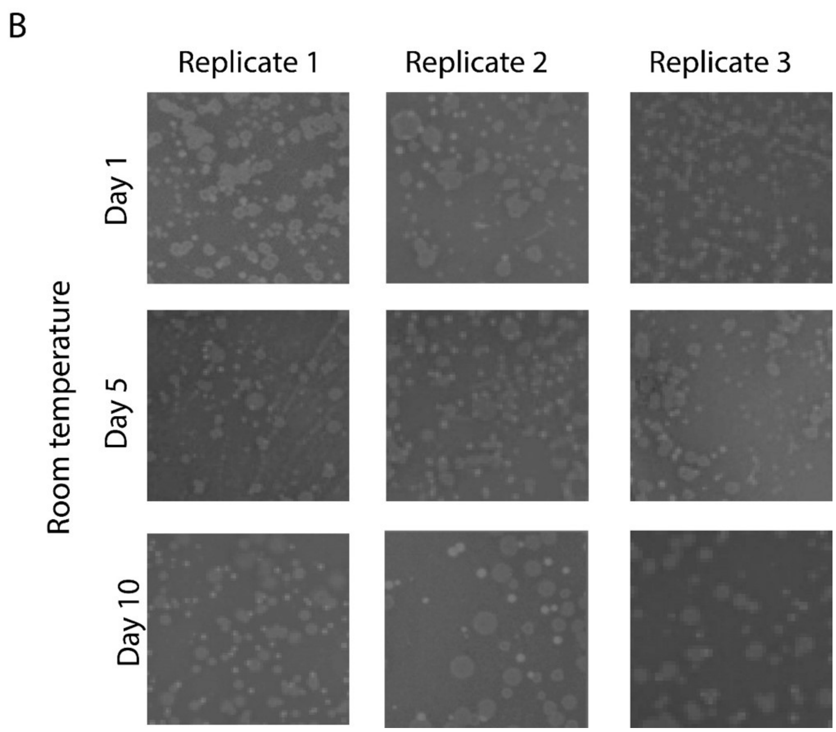


Figure S2. Gel electrophoresis analysis of PCR amplicons from accelerated ageing studies. A specific 185bp band is amplified in all the replicates of accelerated aged samples. Control represents a fresh sample that was not dried. M: Marker; N: No template control; Rep1, Rep2, and Rep3: $1cm^2$ Standard 17 triplicates.

Supplementary Figure S3. Bacterial culture from *Msm*-spiked mock sputum dried on Standard 17 for 10 days at room temperature, 37ºC, and 45ºC.





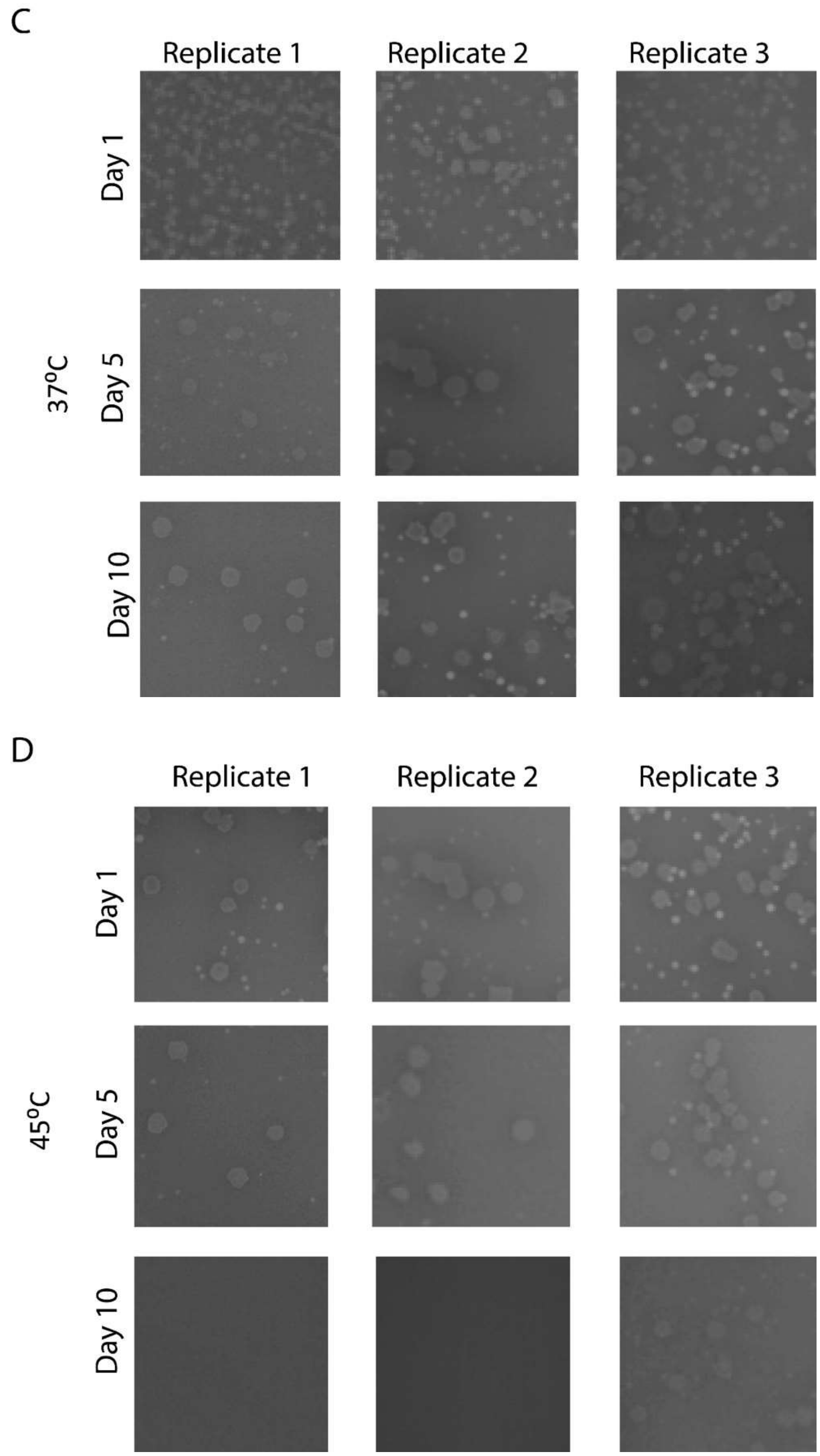


Figure S3. Bacterial culture from dried mock sputum. All plates were stained with ethidium bromide (EtBr) and imaged in a UV gel imaging station. **A**: Controls from fresh samples show that *E. coli* bacteria uptake EtBr while *Msm* do not. Lack of EtBr uptake may be used as a test to confirm *Msm* colonies. **B-D**: Bacterial culture from sputums stored at room temperature (B), 37 ºC (C), and 45 ºC (D).

Supplementary Figure S4. Colony culture PCR from colonies formed from dry stored mock sputum

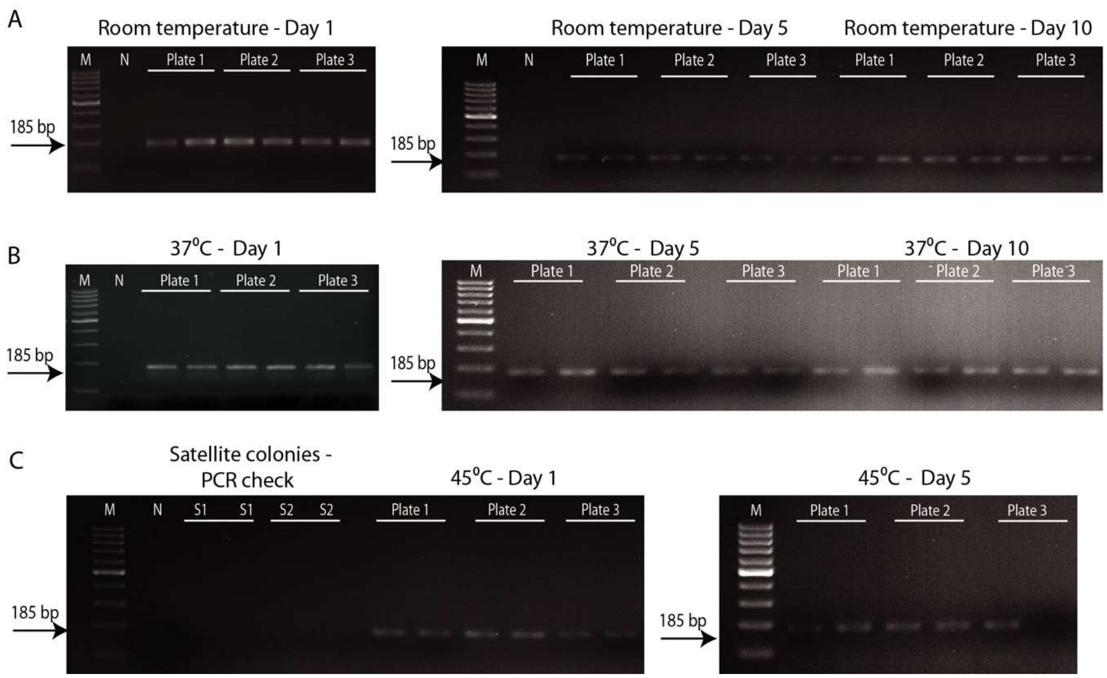


Figure S4. Colony culture PCR. Gel electrophoresis analysis of products of PCR from samples stored at room temperature (**A**), 37 ºC (**B**), and 45 ºC (**C**). Presence of the 185 band confirms that the colonies were formed by *Msm* bacteria. A part of panel **C** shows results from colony culture PCR from satellite colonies. Absence of the 185 bp band confirms that the satellite colonies were not formed by *Msm*.

Supplementary Figure S5. Experimental setup for measuring relative humidity within SPECTRA-Tube

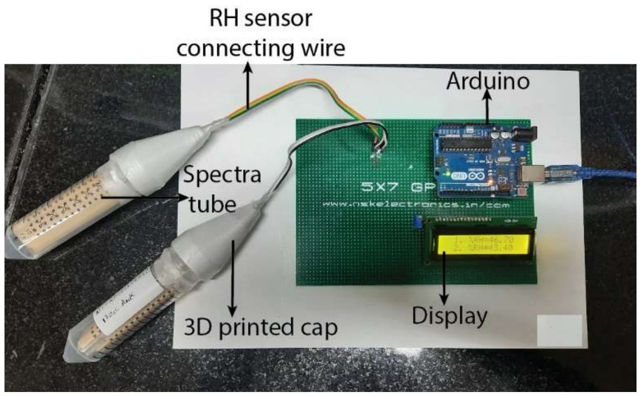


Figure S5. Experimental set-up to measure relative humidity inside SPECTRA-tubes

Relative humidity (RH) was measured inside SPECTRA-tube using a DHT22 AM2303 digital temperature humidity sensor module. The cap of SPECTRA-tube was replaced by an extended 3D printed cap that could held the RH sensor. The tube was perfectly sealed using parafilm after adding the sample for drying. Two RH sensors were connected to an Arduino to simultaneously collect data from two SPECTRA-tubes. The measured values were displayed on a 16x2 inches display as %RH as well as programmed to be logged in a Microsoft excel sheet every 30 seconds.

Supplementary Figure S6-S7. RH as a function of time during drying in SPECTRA-tube

Two types of controls was first conducted: i) a control in which SPECTRA-tube contained only silica gel, without any membrane or external fluid, and ii) controls in which SPECTRA-tubes were loaded with different types of wet membranes, but no silica gel. In control i), when only silica gel was present, the RH dropped rapidly over the first 20-30 min and reached a value of 20% asymptotically at 8 hours (orange curve; Fig. S6). This represents the minimum RH that can be achieved within SPECTRA-tube. In the second set of controls, different 8 cm x 2 cm membranes containing fluid were introduced into SPECTRA-tube, in the absence of silica gel. These controls would report the maximum RH that may be reached in these systems. For a saturated nitrocellulose membrane containing 190 µL water, the RH asymptotically reached a value of 78.7%. Next, 8 cm x 2 cm membranes of filter paper and Standard 17 containing 370 µL water were tested. Filter paper was filled to capacity, while Standard 17 was underfilled to maintain the volume of fluid added constant for the two membranes. Both these conditions generated almost identical RH vs time profiles (light green and dark green curves; Fig. S6), and the RH values reached around 96% for both cases. For an 8 cm x 2 cm membrane of Standard 17 that was saturated with 600 µL water, the RH reached a final value of 97.8% (blue curve; Fig. S6). These results show that the final RH values reached in SPECTRA-tube in the absence of desiccant increase as the volume of fluid introduced increases, as expected.

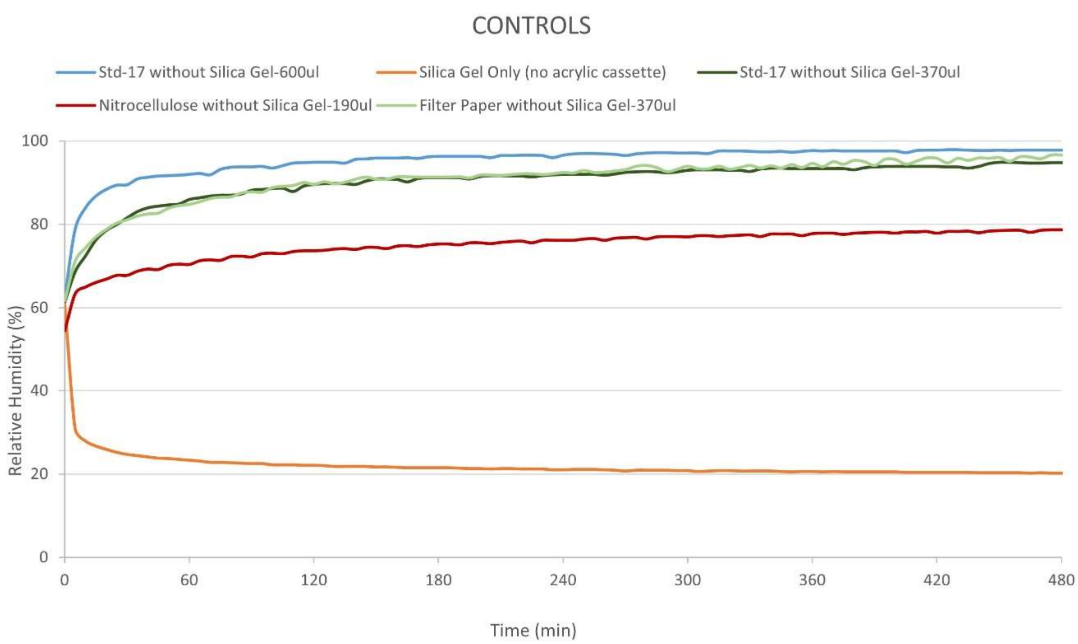


Figure S6. RH vs time during the drying of controls. Orange line represents RH as a function of time with only silica gel in SPECTRA-tube. Other lines represent conditions in which SPECTRA-tube contains only wet membranes, but no silica gel.

In the subsequent set of experiments, SPECTRA-tubes containing silica gel were loaded with three different types of saturated membranes – Standard 17, filter paper, and nitrocellulose, containing 600, 370, and 190 µL water, respectively. Three SPECTRA-tubes were tested for each membrane and RH vs time data was obtained. For all membranes, there was a rapid drop in RH initially (Fig. S7 A-C), within 30 min. This was similar to the shape of the curve obtained for the silica gel only control (orange curve; Fig. S6), which suggests that the rapid initial drop in RH was because of rapid adsorption of the initial moisture in the air. However, after this initial rapid decline, there was either a slight increase in the RH, or the RH values remained constant for some time (Fig. S7 A-C). This phase must represent the phase where moisture is being released from the wet membranes. For almost all cases, near equilibrium conditions were reached in about 3 hours, after which the RH did not change substantially. After 8 hours, the mean (N=3) equilibrium RH values in SPECTRA-tubes containing Standard 17, filter paper, and nitrocellulose were 40.8%, 30.8%, and 29.9%, respectively, and the values for Standard 17 were statistically higher than for filer paper and nitrocellulose (*, N=3, Fig. S7 D). The higher final value of RH for Standard 17 was most likely a result of its higher volumetric capacity, and therefore a higher initial fluid loading in SPECTRA-tube.

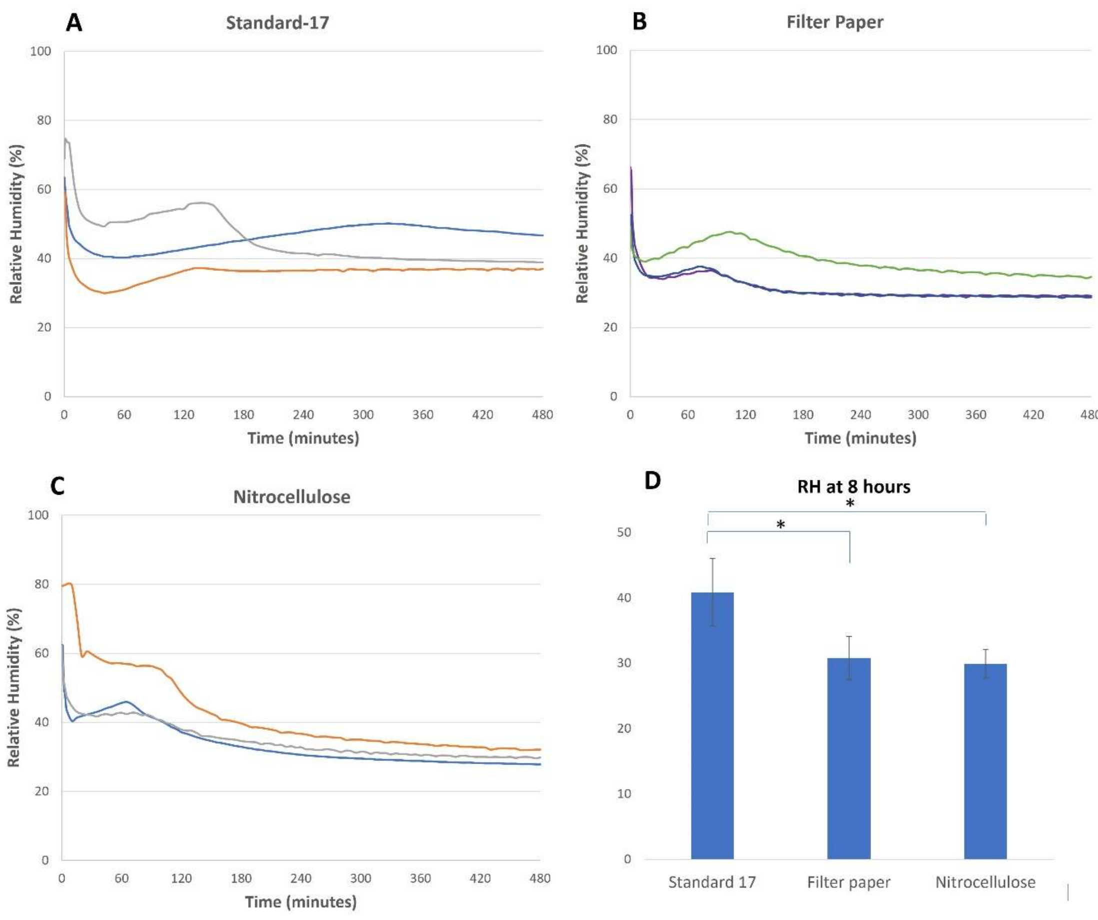


Figure S7. RH vs time during the drying of saturated membranes in SPECTRA-tube. A-C: RH vs time during the drying of Standard 17 (A), filter paper (B), and nitrocellulose (C). D: Mean RH values at 8 hours for the three types of membranes. Error bars represent standard deviation (N=3).

Supplementary Table S1. Optimization of centrifugation speeds for maximizing sample recovery

Sr. No.	Centrifugation Condition		Efficiency (% weight of added sputum that was recovered)
	RPM	RCF	
1.	10,000 for 5 min	16,770 for 5 min	86.20
2.	12,000 for 5 min	24,149 for 5 min	90.05
3.	6,000 for 3 min then 8,000 for 3 min	6,037 for 3 min then 10,733 for 3 min	95.43
4.	6,000 for 3 min then 10,000 for 3 min	6,037 for 3 min then 16,770 for 3 min	95.71

Assumptions

- Radius of centrifuge rotor = 15 cm

All experiments were performed using 2-layer SPECTRA-tube devices in which 1.2 mL mock sputum was introduced. Initially only a single RPM for 5 min was used (Sr. No. 1 and 2; Table S1), but efficiency of recovery was only 90.05% at 12,000 RPM. Rather than increasing the RPM further, a 2-stage recovery was tested comprising of a lower RPM for 3 min followed by a higher RPM for 3 min (Sr. No. 3 and 4). This strategy increased recovery to around 95%.

Supplementary Table S2. Cost of components used in a 2-layer SPECTRA-tube

Component	Required quantity	Units	Cost per unit (INR)	Cost in SPECTRA-tube	
				INR	USD
Corning centrifuge tube	1	tube	31	31	0.42
Standard 17 membrane	32	cm^2	1.09	35.12	0.24
2.6 mm thick acrylic	32	cm^2	0.33	10.56	0.14
Pressure sensitive adhesive	32	cm^2	0.39	12.48	0.17
PDMS tape	16	cm^2	0.45	7.2	0.1
3D printed funnel	5	g	1	5	0.07
TOTAL				**101.36 INR**	**1.38 USD**

Assumptions

- 1 USD (US dollar) = 73.2 INR (Indian Rupee)