

Comparison of smear microscopy, solid culture and liquid culture for monitoring treatment response in pulmonary TB

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the REMoxTB study***

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Study Design

- 186 patients with pulmonary TB and acid fast bacilli (AFB) positive sputum ($\geq 1+$) participating in a double blinded TB drug trial were included
- One sputum sample was collected weekly for the first 8 weeks, followed by week 12, week 17, and week 22
- This study compares three different laboratory methods for monitoring treatment response

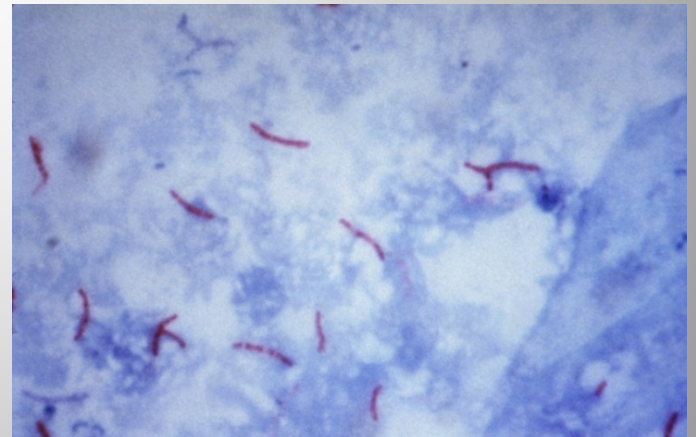
Protocol (I)

Decontamination:

- 20 minutes exposure to 1 % NaOH/N-acetyl-L-cysteine
- Neutralization with phosphate buffered saline (PBS) pH 6.8
- Centrifugation for 15 minutes at 4 °C and 3000 g
- Same Pellet used for all following procedures

Microscopy:

- Smears from 30 µl of concentrated pellet using Ziehl-Neelsen (ZN) staining method



Protocol (II)

Löwenstein-Jensen (LJ) culture:

- Remaining pellet resuspended in 1.5 ml PBS
- 100 μ l used to inoculate one LJ slant
- LJ slant incubated for 8 weeks at 37 °C
- Positive cultures verified by ZN

BD BACTEC™ MGIT960™ system (MGIT):

- One MGIT tube inoculated with 500 μ l resuspended pellet
- MGIT tubes incubated for a maximum of 42 days (1008 hours)
- Positive MGIT cultures tested for contamination on blood agar and verified by ZN



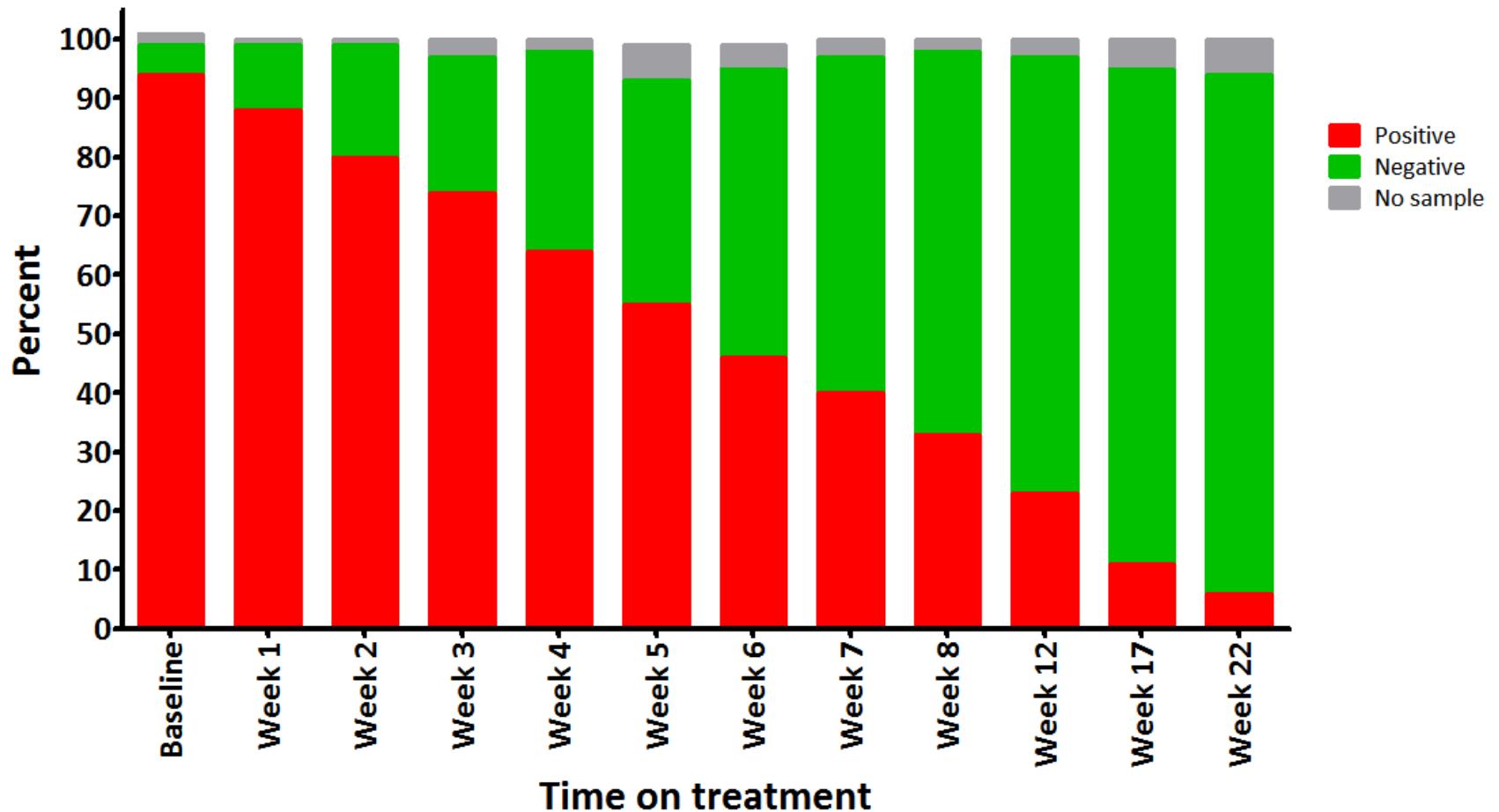
Readout and Analyses

- ZN microscopy slides and LJ cultures were examined by trained technologists and graded according to WHO/IUATLD guidelines

Score	ZN microscopy grades	LJ culture grades
0	No AFB seen/negative	Negative
1	Scanty (< 10/100 fields)	< 20 colonies
2	1+ (< 100/100 fields)	1+ (20 – 100 colonies)
3	2+ (< 10/field)	2+ (101 – 200 colonies)
4	3+ (>10/field)	3+ (>200 colonies)

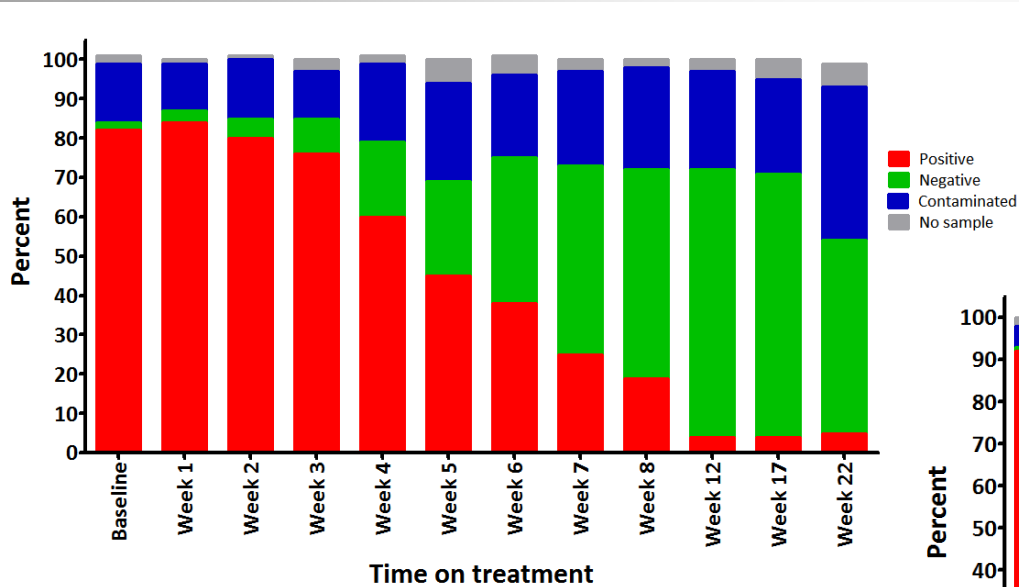
- MGIT tubes were continuously monitored by the automated system for a maximum of 42 days (1008 h). Negative tubes were removed after that time of incubation
- No retreatment data

Qualitative Results of ZN Microscopy

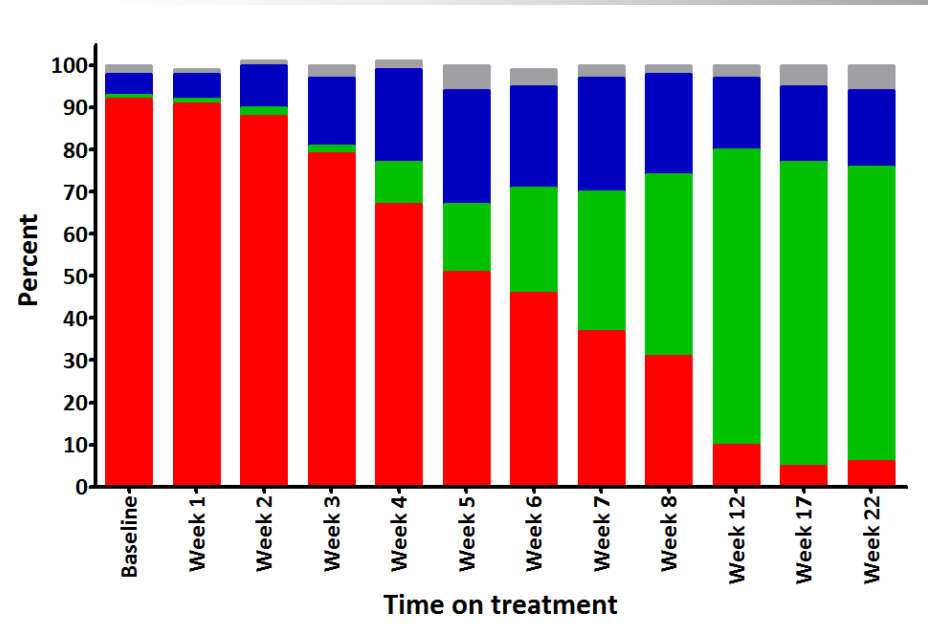


Qualitative Results of LJ and MGIT Cultures

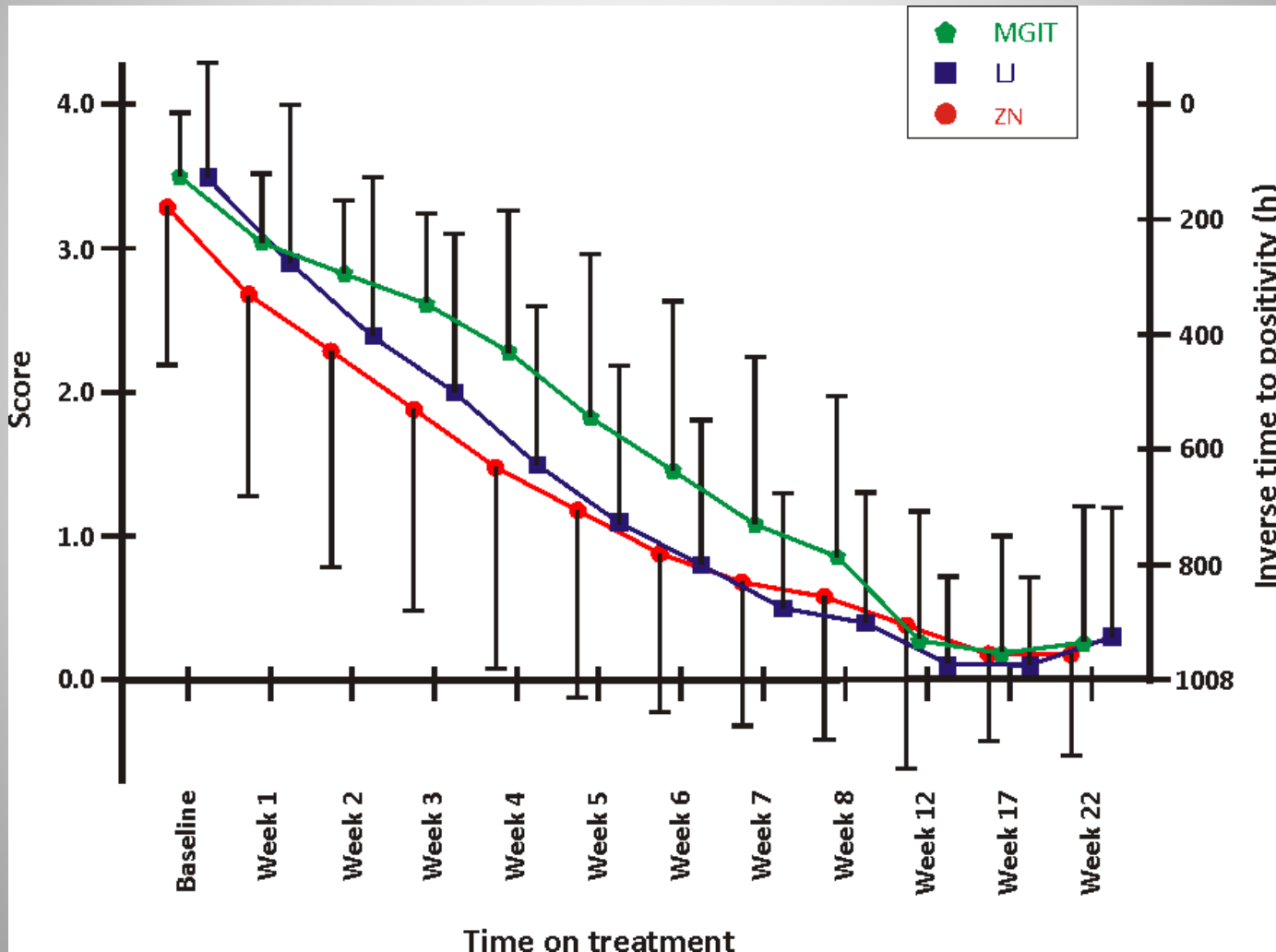
LJ cultures



MGIT cultures



Semi Quantitative Results of All Three Methods

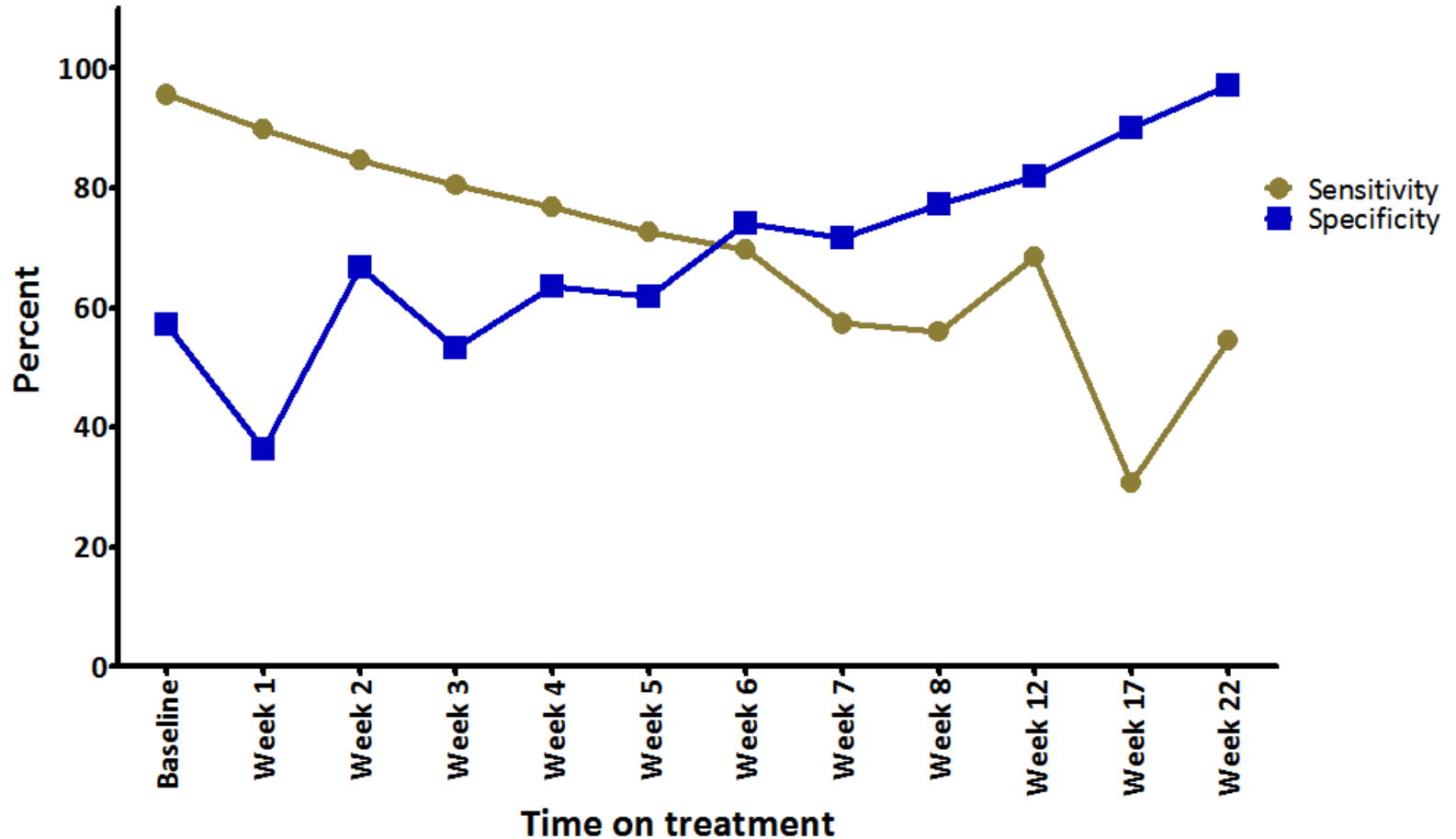


Calculation of Sensitivity and Specificity

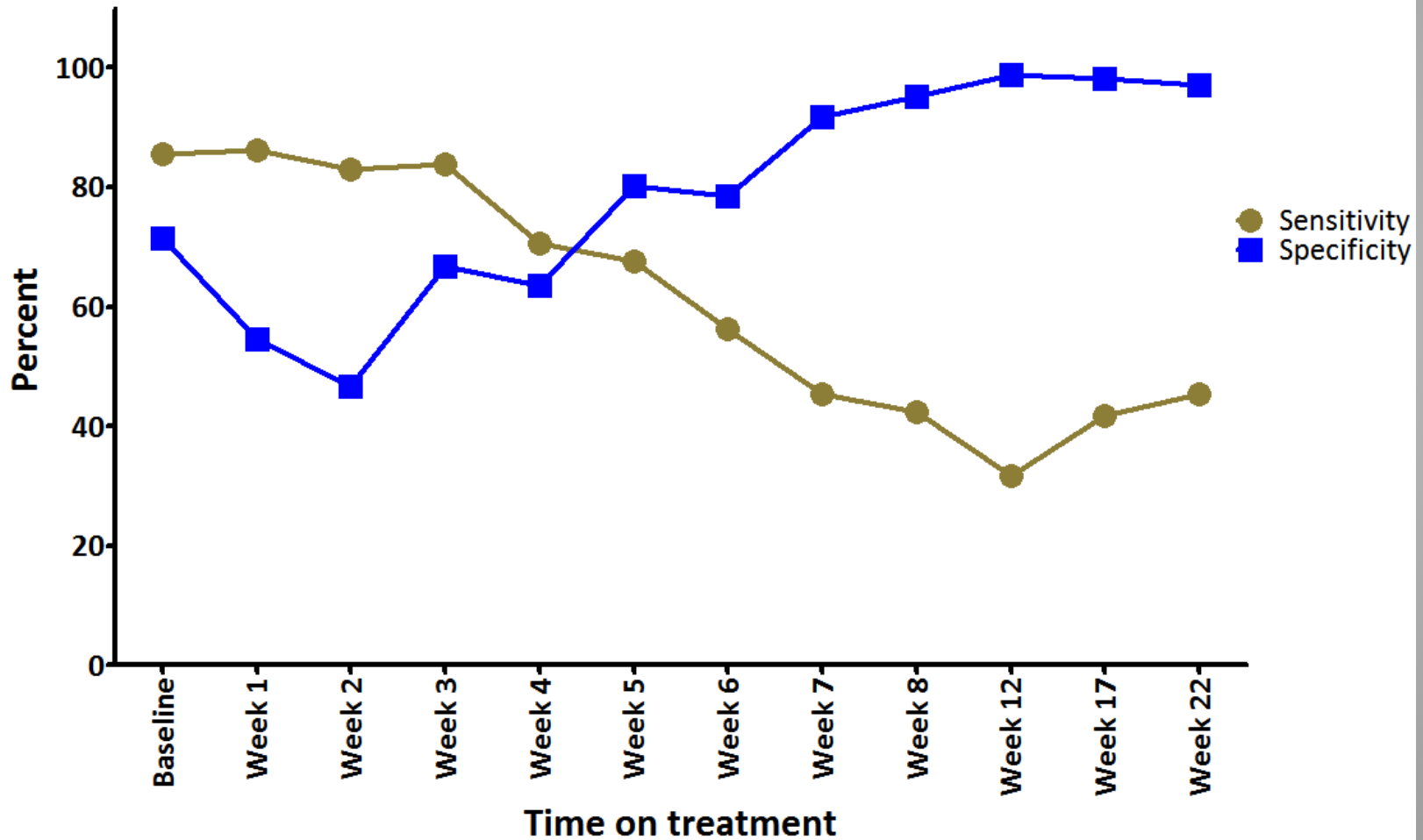
The MGIT culture results were defined as “gold standard” and sensitivity and specificity of ZN microscopy and LJ cultures were calculated

		Gold standard (MGIT)	
		Positive	Negative
Test outcome	Positive	True Positive	False Positive
	Negative	False Negative	True Negative
		↓ Sensitivity	↓ Specificity
		$\frac{TP}{TP+FN}$	$\frac{TN}{TN+FP}$

Sensitivity and Specificity of ZN



Sensitivity and Specificity of LJ



Summary

- All three detection methods show a decreasing load of *Mycobacterium tuberculosis* in sputum under chemotherapy
- The BACTEC™ MGIT960™ system is the most sensitive and it is less susceptible to contamination than LJ culture
- The LJ culture system is the least sensitive but it is more specific than ZN microscopy
- The proportion of non-produced samples is low but the number of contaminated cultures are high

Conclusion

- Regarding sensitivity and rate of contaminations the BACTEC™ MGIT960™ system seems to be the best option for monitoring TB in patients under treatment
- LJ cultures should only be used if comparison to historical results with this method is planned
- The sensitivity of ZN microscopy to detect a positive MGIT culture in the late phase of treatment is relatively poor

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