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Minimum Inhibitory Concentrations: Interpretation and Cross-sectional Analysis in An Unstandardised 7H9 *Mycobacterium tuberculosis* Broth-based System - a Hypothetical Case

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Mycobacterium tuberculosis **is an infectious disease of the lungs and is spread in the air through respiratory droplets. The living state of human beings with tuberculosis infections have become devastating, with particular reference to developing countries such as South Africa, China and India, because of the lack of resources to perform important laboratory procedures like Minimum Inhibitory Concentrations (MICs), which are important to determine the lowest concentration of a particular drug that would ultimately inhibit the multiplication of tuberculosis isolates. MICs thus give scientists an idea as to what diagnosis should be made to treat such patients. This article sheds light and serves to help the scientific community understand and interpret Minimum Inhibitory Concentrations, even where laboratories are under-resourced in the world, and thus contributes to the wealth of scientific, medical, microbiological and public knowledge.**

Key words: Minimum inhibitory concentrations, Susceptibility patterns, Colony counts, Dilution factor, Drug resistance.

The acronym, MIC, stands for the Minimum Inhibitory Concentration. When translated correctly, in microbiology, it is the lowest concentration at which a compound (*viz*. a drug, plant-derived agents or natural products) needs to be administered, in order to hinder the multiplication of a particular type of microorganism or bacteria like tuberculosis. Hence, the term 'administer' gives the definition more specificity by having diagnostic and medicinal connotations (Rishan Singh, personal definition).

Tuberculosis (TB) presents itself, in infected patients, in 3 different phases, namely: primary, secondary and tertiary; and the progression of the severity of TB infection in those patients, depend on the course of treatment that the individuals are taking as well as the time interval to prognosis, from first acquiring the bacterium from the air (Rishan Singh, personal writing).

Tuberculosis, just like the Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS), is a disease pandemic that is easily acquired and spread (Alexander and Strete, 2001) because of its rapid rate of multiplication and thus invasion in the body (as a general term) (Rishan Singh, personal writing).

People, for example, who have reached the tertiary phase of tuberculosis treatment, are often viewed as being less hopeful for survival by themselves, because of treatment failure often ensued in that phase of treatment. Therefore, scientists are switching to the testing of tuberculosis drug combinations in hope of killing and thus ridding the body of this transmissible disease. It is imperative to remember though, that in addition to lung tuberculosis - penile, intestinal,

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and many other types of tuberculosis exist (Rishan Singh, personal writing and preliminary readings). In a country like South Africa where food security, crime, and poverty eradication issues are, most incident and, prevalent, the spread of TB has corresponded with the international Union of Tuberculosis and Lung Disease and the World Health Organisation reports (Singh, Rishan, 2011; WHO, 1997, 2000, 2001). In addition the poor, the unemployed, the homeless, as well as those who have working/family obligations and work in companies that instil strict HIV/TB policies, are more likely to fail primary and secondary tuberculosis treatment (Singh, Rishan, 2011)

The treatment of tuberculosis is first assayed in the laboratory, on tuberculosis isolates acquired from a patient, before a drug or combination of drugs is given to the patient. The one way of testing the effectiveness of a drug against tuberculosis is by performing MICs (Rishan Singh, personal writing). The MIC is a drug susceptibility test that assesses drugs at various concentration ranges and at various time intervals under standard laboratory conditions or modified laboratory conditions, depending on the drug being 'administered' to the tuberculosis bacterial culture (Rishan Singh, personal experience). The method for performing MIC is usually standardised using a MacFarlane Standard. There are a range of MacFarlane Standards that can be used for example, a MacFarlane Standard 1. This 'standard' ensures that all the wells of the tissue culture plate have the same concentration of tuberculosis culture (Rishan Singh, personal writing and experience).

Twenty four well tissue culture plates are used by microbiologists when they are interested in performing MICs using the 7H9 broth-based system. This is the system I am interested in explaining. There are, however, methods in *E.coli* research, for example, where the disc-diffusion method has been reported. The broth microdilution method is a fast but labour intensive technique if one is to use it to perform MICs in research (Rishan Singh, research experience).

The second-line anti-tuberculosis drug, cycloserine, has become scarcely reported in tuberculosis research lately. Much of this rareness is due to its high toxicity, and the adverse effects it invokes, on TB-infected patients (WHO PAR Part 4, 2007). The high toxicity of cycloserine has indicated that it has a divergent role in neurology (Wolinsky, 1993; Rishan Singh, personal writing). This secondary function of cycloserine has to be eliminated if it is to be re-introduced as an effective anti-tuberculosis drug, however this can only be achieved by reducing its toxicity while maintaining effective dosages, which is difficult to achieve even to pharmaceutical chemists and technologists (Rishan Singh, personal laboratory deduction). This makes the study of MICs an imperative for drug compounds like cycloserine, and several others which are associated with some form of instability, so as to reintroduce 'old TB drugs' back into treatment trials and to avoid the costs involved in manufacturing newer 'better' ones. This would allow the creation of gold standards for those TB drugs (Rishan Singh, personal writing, 2011). For the cross-sectional analysis and explanation of MIC results that follow, we would assume that cycloserine was administered at concentrations: 8, 16, 32, 64 and 128 µg/ml to MDR-TB isolates in the wells of the 24-well tissue culture plate and read after 7, 14, 21 and 28 days. The exact method of performing MICs will not be explained because this article serves to demonstrate the point of drawing conclusions from MIC results (Rishan Singh, personal writing).

MICs can be read on the day that one desires to, but most scientists throughout the world choose to read an MIC on the day which previous studies and methodologies report for different drug compounds and isolates of tuberculosis (Rishan Singh, personal writing and experience).

To create a hypothetical case and, make a study to serve as a reference study for other scientists to use throughout the world, I chose to be 'creative' by using the second-line anti-tuberculosis drug, cycloserine. I made this selection on the basis that cycloserine is unstable in tuberculosis studies using the broth microdilution method (Martin-Casabona *et* al., 1997; Victor *et* al., 1997; Rishan Singh, personal writing). This report focuses more on the way of analysing drug susceptibility test results in an unstandardized MDR-TB mycobacterial system without giving too much consideration to the instable nature of cycloserine. This article is written only to serve as a reference standard that can be used to analyse MICs and/or drug susceptibility results. Furthermore, the results were analysed taking into

account that 10^4 dilution of the mycobacteria culture was optimal. However, in the laboratory this differs on the basis of the McFarlane standard that one would chooses to use. I will now analyse these hypothetical MIC results in the form as if it was truly performed in a third world laboratory setting (Rishan Singh, personal writing and deductions).

Table 1. Minimum Inhibitory Concentration (MIC) results for 42 MDR-TB isolates compared to their respective MIC results of the control, $H_{37}R_v$. Brackets [ ] denote isolates tested on the same day

MIC (µg/ml) (day 21)	Total no. of isolates	%	Isolate nos. correlating with the MIC of $H_{37}R_v$	%	Isolate nos. not correlating with the MIC of $H_{37}R_v$	%
8	0	0	-	-	-	-
16	0	0	-	-	-	-
32	20	48	[3, 4]; [5, 6, 7, 8]; [14, 15]; [18, 21]; [32, 33]; [34, 35, 36, 37*]; [39, 40, 41, 42]	100	0	0
64	16	38	[9]; [11, 12]; [22, 23, 24]	38	[1,2]; [19, 20]; [26, 27, 28]; [31]	50
>64	6	14	0	0	[1, 3]; [10]; [29, 30]; [38]	100

*atypical growth pattern

A total number of 42 isolates were tested and of these, 20 isolates had an MIC value of 32 µg/ml, 16 isolates had an MIC value of 64 µg/ml, and 6 isolates had an MIC value of more than 64 µg/ml. All 20 isolates (100%) that had an MIC value of 32 µg/ml correlated with the MIC of the control strain, $H_{37}R_v$, with an exception of isolate 37 which I classified as being atypical due to an inconsistent growth pattern across the susceptibility period. Of the 16 isolates that had an MIC of 64 µg/ml, 38 % ($^6/_{16}$) of them had MIC values that correlated with the MIC of $H_{37}R_v$, while none (0 %) of the 6 isolates that had MIC values of > 64 µg/ml correlated with the MIC of the control strain i.e. 100 % of them had MICs that did not correlate with the MIC of $H_{37}R_v$, on the respective days of reading the MIC at 21 days. These isolates were: 10, 13, 25, 28, 30 and 38. However, 50% ($^8/_{16}$) of the isolates with MICs of 64 µg/ml had MICs that did correlate with the MIC of $H_{37}R_v$ (1, 2, 19, 20, 26, 27, 28, and 30).

I classified isolate 37 as being atypical because on the day of reading the MIC, the plate exhibited growth in the wells containing 16 µg/ml and 64 µg/ml of cycloserine. This result clouds my vision of MICs because in order for an MIC reading to be valid, no growth should be present in all wells after the MIC well is read at day 21. When one encounters such a significant observation when reading MIC results, colony counts become important. One can rule out the possibility of this being due to inappropriate colony counts if the MIC results of many other MDR-TB tested isolates, are valid having the same colony count as the atypical isolate (see 2×10^7; 1, 2, 3, 4, 5, 7, 9, 12, 14, 16, 19, 21, 24, 28, 30, 33, 34, 36, 37, 40, 41, and 42). Furthermore this could not have been ascribed to pipetting errors of the drug into the 64 µg/ml well, because the experiment represents results that are in triplicate and the same results were obtained for all 3 sets of results (given the results in Table 1). It can be suggested that the isolate be genetically tested because the phylogenetics of moving from a tuberculosis isolate that is susceptible, (consume the drug) to a drug compound like cycloserine, to one that is resistant (does not take consume the drug) to the same compound, is not mediated by the plasmid insertion of resistant genes (Petrini and Hoffner, 1999). MDR-TB, instead, arises and results from the pressure imposed on and experienced by tuberculosis isolates, resulting in genetic mutations

in the bacterium and thereby contributing to the virulence of the bacterium; its spread, infection and disease (Petrini and Hoffner, 1999; Rishan Singh, personal writing). This makes the study of MICs imperative in poverty stricken countries like South Africa, where laboratory resources are undermined by the huge costs involved in performing MICs of good accuracy and precision (Rishan Singh, personal writing). However, it is important to note that results like that of the atypical isolate creates ambivalence to the scientific community worldwide and can be considered a milestone finding in tuberculosis research (one which I have discovered). Therefore, we must not lose hope in the challenges that we face in carryout MIC tests since such results are not due to the misapprehension of the scientists/ microbiologists performing these drug susceptibility tests, but rather to limited resources (Rishan Singh, personal writing, observation and experience).

As it stands these individual patient isolates appear to be representing the same patient, but this is not true because, each patient will have a different treatment regimen and be treated differently (Rishan Singh, personal writing). Therefore, from this, the complete treatment profile needs to be given so that scientists can be made aware of the treatment regimens such patients are on (Rishan Singh, personal writing). Twelve out of the 42 MDR-TB isolates showed a single shift in the MIC reading from day 7 to 14 to 21 (29 %; 7, 9, 17, 20, 22, 23, 24, 26, 27, 28, 31, and 32), while the remaining 71 % had MIC values that were repetitive, at some days, across the 4 week susceptibility period; this value including the atypically classified isolate, number 37. However, looking at the day 28 drug concentration that possibly instigates inhibition to some degree, a remarkable, interesting, but not surprising discovery is that of the 29 % of isolates that showed a single shift in MIC values, in that 83 % ($^{10}/_{12}$) had readings at day 28 that correlated with the MIC value on 21 days. The majority of these had colony counts between $1 \times 10^7 - 3 \times 10^7$, with an exception of isolate 31, which had colony counts of 6×10^6. This is an important deduction because it indicates that colony counts had little or no effect on the outcome of the drug susceptibility result for day 28, i.e. all of them being 64 µg/ml (Rishan Singh, personal deductions from hypothetical scenario and laboratory research).

Isolate 61 was interesting because it had an MIC of 64 µg/ml with a low colony count compared to its counterparts. Isolates 7 and 9 had the same colony count (2×10^7), but their MICs were 32 and 64 µg/ml respectively, while when isolates 17, 21, 23 and 27 are compared to isolates 9, they exhibit the same susceptibility pattern but they are present at 3×10^7 colony forming units per millilitre (cfu/ml). The question that arises from these results are: 'at what cfu/ml would be performing MIC tests optimal?' (if such an optimal exists) (Rishan Singh, personal deduction and writing). These statements are further reinterated by the fact that isolates 24 and 28 do not have MIC values that are analogous to those of isolates 7 and 32 that have the same colony counts of 2×10^7 cfu/ml (Rishan Singh, personal writing). We know the significance of colony counts is to optimise and standardise the techniques used to determine MICs and to get uniform cell culture results, but now we can ponder their relevance, even though we know that different patients are on different treatments regimens. In such a study where the susceptibility profile of individual patient isolates are absent, it is difficult to make proper conclusions using cfu/ml as a set point, because it is possible for some of these patients, to be on combination therapies or utilising other form of treatment options (Rishan Singh, personal writing and definition in context of article).

$H_{37}R_v$ for isolates 14 and 15 show an MIC of 32 µg/ml at day 21. When one looks at the cell count of this control strain, 3×10^6 cfu/ml, it is possible to deduce that the colony count is the reason for there being no shift in the MIC reading at day 28 – reason, the cell count of this control strain is relatively low when compared to the $H_{37}R_v$ isolates used, tested and compared with all the other isolates except for isolates; 31, 32, 33, 34, 35, 36, 37 and 38. The cfu/ml of the $H_{37}R_v$ strain for these 2 set of MDR-TB isolates tested, were 2×10^6 and 3×10^5, respectively. The latter value confirms that although the colony counts are important in performing MICs and/or drug susceptibility tests, the switch in drug concentrations that affects the multiplication machinery of tuberculosis bacterium, does not depend on the amount of bacterium being used (Rishan Singh, personal innovative discovery).

Table 2. $H_{37}R_v$ isolates showing the same drug, cycloserine, concentrations at days 21 and 28 (arranged in ascending order of colony counts)

cfu/ml	Key	7	14	21	28
2×10^6	*K*	8	16	32	32
3×10^6	*F*	8	16	32	32
8×10^6	*D*	16	32	64	64
8×10^6	*E*	16	32	64	64
1×10^7	*C*	8	16	32	32
1×10^7	*M*	8	16	32	32
2×10^7	*I*	16	32	64	64

From the table above, one would expect that as the cfu/ml increases from 2×10^6 to 2×10^7 for $H_{37}R_v$ (in the order: *K*, *F*, *D*, *E*, *C*, *M* and *I*), the concentration of cycloserine required to hinder bacterial multiplication would be high. However, this is not the case. $H_{37}R_v$ strains *K*, *F*, *C* and *M* exhibit the exact same susceptibility pattern across the four week period with a 'precise' MIC at day 21. The MIC of these isolates are considered precise because once the concentration of drug inhibiting TB growth was established, at day 21 (32 µg/ml) the same reading persisted at day 28 and this was an indication of maximised external and internal validity (Rishan Singh, personal result analysis deduction).

The McFarlane is a turbidity standard, as mentioned, that enables microbiologists to plate out a particular concentration of microorganism (Rishan Singh, personal conclusion). It is believed that a dilution factor of 10^4 would achieve colony counts of 1×10^7 cfu/ml. As mentioned, *K*, *F*, *C* and *M* show the same susceptibility profile, with *C* and *M* giving more accurate colony counts, but *D* and *E* have the same colony counts (8×10^6 cfu/ml) and show the same susceptibility pattern to the drug as it were $H_{37}R_v$ (I), present at 2×10^7 cfu/ml (Rishan Singh, personal observation). To many scientists, professors and other senior officials in laboratories and institutes throughout the world, these MIC results of $H_{37}R_v$ relative to the tested MDR-TB results may not be valid, but I would explain as to how and why they are valid and of universal importance. The fact that *G* and *J* have the same colony counts as *C* and *M*, which we consider optimal, but have a single shift in the concentration of the drug inhibitory growth across the four week susceptibility period, it is an indication that there is no established link between an established MIC reading and the colony count (Rishan Singh, personal conclusion)

Table 3. $H_{37}R_v$ isolates, showing a poor representation of MIC results, with the same drug, cycloserine, concentrations at days 7 and 14 or days 14 and 21 (arranged in ascending order of colony counts)

cfu/ml	key	7	14	21	28
3×10^5	*L*	16	32	32	>64
7×10^6	*B*	8	32	32	32
8×10^6	*A*	16	32	32	64
4×10^7	*H*	16	16	32	64

Four $H_{37}R_v$ isolates (*L*, *B*, *A*, *H*) represent a poor representation of MIC results on its own i.e. when not compared to the treated MDR-TB isolates, because neither one of them show an MIC that started at day 21 and remained constant at day 28. However, the most accurate MIC representation from these four is isolate *B*, because it retained its MIC value after 21 days. Conversely enough, *L*, *A* and *H* indicates that the mycobacterium requires a higher concentration of drug to cause inhibition because it does not retain its MIC value after 28 days of treatment even though the MIC value may be the same (*L* and *H*) or not (*H*) at 14 days. $H_{37}R_v$ (*H*) exhibits interesting susceptibility patterns with the concentration of the drug being responsible for inhibiting mycobacterium growth by 7 and 14 being the same. The reason could be that the drug contributes to drug resistance in the bacterium by 21 days, but is less resistant at day 7 and 14 so as to be inhibited by only 16 µg/ml of the drug. Human error such as incorrect pipetting of the drug could also contribute to the resistance seen in *H*. However, in *L*, *B*, *A* and *H*, there is no established link between the colony counts and MIC patterns and this holds true in conjunction with all of the other $H_{37}R_v$ isolates, with much of the confusion arising from isolates *H*, *G* and *J* (Rishan Singh, personal conclusions and writing).

In a Southern African context, and possibly also in other parts of the world, traditional and herbal medicines are believed to be of great significance to some, and with the faith that some have in those remedies, the infestation of MDR-

TB or TB, in general, is believed to become reduced in such people. I am uncertain as to whether these cures really do work, but if they do, it could explain the reason for there being such variability in MIC results for individual patient isolates. Furthermore, depending on the stage at which these patients are treated, one can expect colony counts to be insignificant if the stage of tuberculosis diagnosis and prognosis is unknown. Some patients, from the study, may have been treated so late that MDR-TB was at the stage of changing phylogenetically into possibly XDR-TB; emphasised by the susceptibility pattern observed for isolates; 10, 25, 29 and 38, all of which have different colony counts (Rishan Singh, personal writing and deductions). The idea of these four isolates requiring such a high concentration of cycloserine (64 µg/ml) or more to inhibit growth in the case of isolate 10 on the 8th day, suggests that this is possibly due to the immunosuppressed state of the patient (Alexander and Strete, 2001), perhaps due to late tuberculosis treatment. Also, such a patient may have other reasons for presenting themselves for treatment so late like financial, economic, social and sexual reasons. These patients could also have other health problems like HIV co-infection or even AIDS or other Sexually Transmitted Diseases (STDs), and could be shy to open up and speak about it in front of a practitioner or specialist consultant, as this is a commonly known introverted state of these infectious diseases (Singh, Rishan, 2011; Rishan Singh, personal writing).

Isolates 2, 5, 6, 8, 11, 12, 29, 40 and 41 showed the same drug inhibitory concentration for days 7 and 14. These readings do not say much about drug resistance when compared with the day 21 MIC values, but when looked in-depth and in isolation in terms of interpreting it as day 7 and 14 only, it indicates that the isolates, MDR-TB, have different susceptibilities and resistances to cycloserine and that the repetitive MIC value at day 14 as day 7 (before the increase at day 21), indicates some level of growth inhibition of the mycobacteria by a lower concentration of the drug. This is an important scientific breakthrough and raises the bar for interpreting MICs or drug susceptibility patterns (Rishan Singh, personal writing, deductions and conclusions).

Isolates 26 and 30 are important result findings that have hidden subtleties. Isolate 26 is a 'made-up' isolate result to show more in-depthly how to interpret MICs. As it stands it appears as being unclassified and uninterrupted. It could have the reasons as isolate 37 i.e. have meaning that on day 14 the wells containing 16 µg/ml and 32 µg/ml had tuberculosis growth, or we could postulate that the MIC on day 14 would be 32 µg/ml because more than 60 % ($^{29}/_{42}$) of all the isolates tested had MICs of 32 µg/ml. However, we must keep in mind that the treatment regimen of patient 26 is not known and therefore such a deduction can't be made when one looks at the set of isolates tested only [26, 27, 28, 29 and 30] (Rishan Singh, personal conclusions and deductions). Interestingly though is the result of isolate 30 across the four week susceptibility period. This isolate initially required 16 µg/ml to slow down the rate of mycobacteria multiplication by the 8th day, with a 4-fold concentration of cycloserine being required by day 14. This could have been due to an increase in the resistance of the MDR-TB isolate to cycloserine or it could also mean that the mycobacteria for that patient mutated by day 14, causing it to require a higher concentration of cycloserine to be inhibited (Rishan Singh, personal deduction and conclusion).

In all cases of drug-resistant tuberculosis, resistance emanates from the fact that rod-shaped mycobacterial cell wall constitutes 60 % lipids, which is made up of mycolic acids, cord factor and wax-D, apart from the peptidoglycan fraction (Todar, K, 2011). However, it is the mycolic acids in the lipid that make the treatment of tuberculosis a daunting task because they are alpha-branched hydrophobic lipids that affect the permeability properties at the cell surface by preventing drugs from being taken up (Todar, K, 2011). In general, it is a lipid layer that contributes to the virulence of the bacteria (aggressiveness and invasion) and therefore research has to be done to design drugs that can cross the lipid barrier of these mycobacteria allowing them to be phagocytosed by macrophages instead of allowing their survival in macrophages as facultative intracellular parasites (Todar, K, 2011; Rishan Singh, personal reading and adapted writing, 2012).

Humans are the etiological agent of *Mycobacterium tuberculosis,* a large no-motile rod-shaped bacterium which is usually inhabited in

the well-aerated upper lobes of the lungs. The bacteria itself has a slow generation time of between 15 – 20 h which is believed to be the major contributor to the invasiveness and aggressive spread of the bacterium (Todar, K, 2011). In order to combat this spread of tuberculosis across nations, researching and interpreting MIC results is important especially in developing countries like South Africa, Hong Kong, China, Japan and India, where obtaining standardised MIC results are not possible just like in developed countries like Europe, United States of America, United Kingdom and the United Arab Emrites, because of the different stages at which people present themselves for tuberculosis treatment and the treatment regimen administered (Rishan Singh, personal writing). However, the situation in developing countries is worse due to resource limitations and the challenges that the people face. This article is important because it is the first paper, worldwide, that looks at a means of interpreting significant and unstandardised results on drug susceptibility testing results / MICs using a multivariable complex analysis approach. It can be used as a means of interpreting MICs in standardised or unstandardized systems involving any microorganism and any drug compound (i.e. plant-derived or natural product) (Rishan Singh, personal writing).

Appendix Table A: MIC results of cycloserine for individual isolates (including the controls, $H_{37}R_v$) at pH 7.2 (experiments were carried out in triplicate)

Number / Key	Experiment no.	7	14	21 (MIC)	28	Colony counts (cfu/ml) 10^{-3}	10^{-4}
1.	R.10.	32	64	64	>64	7×10^6	2×10^7
2.	R.12.	32	32	64	64	4×10^6	2×10^7
A.	$H_{37}R_v$	16	32	32	64	1×10^6	8×10^6
3.	R.15.	8	32	32	32	5×10^6	2×10^7
4.	R.19.	16	32	32	32	8×10^6	2×10^7
B.	$H_{37}R_v$	8	32	32	32	5×10^6	7×10^6
5.	R.20.	16	16	32	64	7×10^6	2×10^7
6.	R.21.	16	16	32	64	7×10^6	4×10^7
7.	R.23.	16	32	32	64	7×10^6	2×10^7
8.	R.25.	16	16	32	64	1×10^7	4×10^7
C.	$H_{37}R_v$	8	16	32	32	5×10^6	1×10^7
9.	R.39.	16	32	64	64	7×10^6	2×10^7
10.	R.42.	>64	>64	>64	>64	9×10^6	6×10^7
D.	$H_{37}R_v$	16	32	64	64	5×10^6	8×10^6
11.	R.31.	32	32	64	64	9×10^6	4×10^7
12.	R.33.	32	32	64	64	7×10^6	2×10^7
13.	R.34.	32	64	>64	>64	7×10^6	4×10^7
E.	$H_{37}R_v$	16	32	64	64	4×10^6	8×10^6
14.	R.36.	16	32	32	64	1×10^7	2×10^7
15.	R.3.	16	32	32	64	1×10^7	3×10^7
F.	$H_{37}R_v$	8	16	32	32	4×10^6	3×10^6
16.	R.1.	32	32	64	64	7×10^6	2×10^7
17.	R.41.	32	32	64	64	2×10^7	3×10^7
G.	$H_{37}R_v$	8	16	32	64	8×10^6	1×10^7
18.	R.22.	16	32	32	64	1×10^7	2×10^7
19.	R.26.	16	32	64	64	2×10^6	3×10^7
20.	R.27.	16	32	64	64	2×10^7	3×10^7
21.	R.29.	16	32	32	64	1×10^7	2×10^7
H.	$H_{37}R_v$	16	16	32	64	1×10^7	4×10^7
22.	R.17.	16	32	64	64	1×10^7	4×10^7
23.	R.18.	16	32	64	64	8×10^6	3×10^7
24.	R.13.	16	32	64	64	9×10^6	2×10^7

25.	R.16.	64	64	>64	>64	7×10^6	3×10^7
I.	$H_{37}R_v$	16	32	64	64	8×10^6	2×10^7
26.	R.7.	16	*	64	64	7×10^6	1×10^7
27.	R.46.	16	32	64	64	7×10^6	3×10^7
28.	R.0.	16	32	64	64	8×10^6	2×10^7
29.	R.47.	64	64	>64	>64	7×10^6	3×10^7
30.	R.2.	16	64	>64	>64	7×10^6	2×10^7
J.	$H_{37}R_v$	8	16	32	64	8×10^6	1×10^7
31.	R.50.	16	32	64	64	4×10^6	6×10^6
32.	R.6.	8	16	32	64	7×10^6	2×10^7
33.	R.8.	16	32	32	64	7×10^6	2×10^7
K.	$H_{37}R_v$	8	16	32	32	3×10^6	2×10^6
34.	R.32.	16	32	32	64	8×10^6	2×10^7
35.	R.49.	16	32	32	64	5×10^6	1×10^7
36.	R.48.	16	32	32	64	6×10^6	2×10^7
37.	R.27.	16	32	32	*	7×10^6	2×10^7
38.	R.33.	64	64	>64	>64	5×10^6	1×10^6
L.	$H_{37}R_v$	16	32	32	>64	4×10^5	3×10^5
39.	R.51.	16	16	32	64	7×10^6	4×10^7
40.	R.52.	16	16	32	64	7×10^6	2×10^7
41.	R.54.	16	16	32	64	7×10^6	2×10^7
42.	R.55.	16	32	32	64	7×10^6	2×10^7
M.	$H_{37}R_v$	8	16	32	32	5×10^6	1×10^7

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