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# Prediction of hepatitis B virus lamivudine resistance based on YMDD sequence data using an artificial neural network model

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**ABSTRACT**

*Background:* Hepatitis B virus (HBV) infection is an important health problem worldwide with critical outcomes. The nucleoside analog lamivudine (LMV) is a potent inhibitor of HBV polymerase and impedes HBV replication in patients with chronic hepatitis B. Treatment with LMV for long periods causes the appearance and reproduction of drug-resistant strains, rising to more than 40% after 2 years and to over 50% and 70% after 3 and 4 years, respectively.
*Objectives:* Artificial neural networks (ANNs) were used to make predictions with regard to resistance phenotypes using biochemical and biophysical features of the YMDD sequence.
*Patients and Methods:* The study population comprised patients who were intended for surgery in various hospitals in Tehran-Iran. An ACRS-PCR method was performed to distinguish mutations in the YMDD motif of HBV polymerase. In the training and testing stages, these parameters were used to identify the most promising optimal network. The ideal values of RMSE and MAE are zero, and a value near zero indicates better performance. The selection was performed using statistical accuracy measures, such as root mean square error (RMSE), coefficient of determination (R2), and mean absolute error (MAE). The main purpose of this paper was to develop a new method based on ANNs to simulate HBV drug resistance using the physiochemical properties of the YMDD motif and compare its results with multiple regression models.
*Results:* The results of the MLP in the training stage were 0.8834, 0.07, and 0.09 and 0.8465, 0.160.04 in the testing stage; for the total data, the values were 0.8549, 0.115, and 0.065, respectively. The MLP model predicts lamivudine resistance in HBV better than the MLR model.
*Conclusions:* The ANN model can be used as an alternative method of predicting the outcome of HBV therapy. In a case study, the proposed model showed vigorous clusterization of predicted and observed drug responses. The current study was designed to develop an algorithm for predicting drug resistance using chemiophysical data with artificially created neural networks. To this end, an intelligent and multidisciplinary program should be developed on the basis of the information to be gained on the essentials of different applications by similar investigations. This program will help design expert neural network architectures for each application automatically.



▸ *Implication for health policy/practice/research/medical education:*
One of the main obstacles of effective oral antiviral treatment regimens for patients with HBV is viral resistance against oral medications. We suggest reader's attentions in the field of gastroenterology and liver diseases to the conclusion of this article.

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## Background

Infections and cancers that are caused by hepatitis B virus (HBV) are important worldwide health problems with criti-

cal outcomes (1). For persons who are chronically infected with HBV, there are two therapeutic approaches that are used to control the infection and its consequences: immunomodulatory agents and antiviral chemotherapy (2). The nucleoside analog lamivudine (LMV) is the optimal therapeutic choice; it inhibits HBV polymerase and slows HBV replication in patients who are chronically infected with hepatitis B (1-5). Drug resistance remains a global public health problem (6), and resistance to LMV is emerging (2). This phenomenon is mediated primarily by mutations in the genes of viruses that alter a drug's interaction with its corresponding target protein (6). Antiviral drug resistance depends on the frequency of viral mutations, the intrinsic mutability of the antiviral target site, and the magnitude and rate of viral replication (5). Typically, mutations in the YMDD motif of the polymerase gene develop after the first 6 months of treatment (7, 8). Long-term therapy with LMV induces the emergence and propagation of drug-resistant isolates, rising to more than 40% after 2 years and to over 50% and 70% after 3 and 4 years, respectively(1, 2, 7). Lamivudine resistance in HBV is a major clinical brake to its long-term use (9).To avoid exacerbation of the infection, the prediction and detection of lamivudine-resistant viruses are vital processes in clinical management (1). Since structural information is available for only a small percentage of proteins, methods for the direct prediction of resistance, based on viral sequences, are highly desired (6). Lamivudine-resistant viruses have a characteristic amino acid substitution in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of its RNA-dependent DNA polymerase. Several studies have reported various mutations that are induced by lamivudine therapy (7, 8, 10, 11). These mutations in the YMDD motif are necessary and sufficient to confer high-level lamivudine resistance (11). It is important to detect the YMDD motif mutations during lamivudine treatment (10). When mutations occur, the configuration of the wild-type YMDD motif becomes altered such that the drug no longer exerts its inhibitory action at that site (2). Numerous techniques have been introduced to monitor HBV drug resistance, such as oligonucleotide chips (12), line probe assay (13), light cycler probe hybridization assay (14), polymerase chain reaction with peptide nucleic acid clamping (15), mass spectrometry of oligonucleotide fragments (16), fluorescence polarization, and sequencing (17). Most of these techniques are accurate but time-consuming, labor-intensive, and difficult to adapt to high-throughput screening. To be used as a clinical evaluation tool and reduce the cost of therapy, methods that distinguish responders from nonresponders and predict outcomes of the treatments must be established (18). Recently, computer-based models have been used by health care providers for management purposes. Neural networks can solve clinical problems based on symptoms and patterns (19). Drug resistance is a complex phenomenon for which several mechanisms are responsible (6). The progress in informatics and its application in decision-making has led to the development of novel artificial intelligence techniques, including artificial neural networks (ANNs) (20).The ANN has been applied in various disciplines of science and technology (21). Statistical learning methods, such as neural networks (22-28), support vector machines (SVMs) (22, 24, 25, 29, 30), and decision rules (22-24, 27, 29, 30), have also shown potential in predicting resistance mutations. An important application of the ANN is the prediction of responses across heterogeneous domains (21). This study performs a novel examination of the use of biochemical and structural information and neural processing in the context of HIV drug resistance (28).ANNs learn by an iterative process that adjusts the strengths of connections, such that the system generates an appropriate result. Notably, data processing by these systems does not require assumptions of how outputs relate to inputs or that inputs be independent (31). Pattern recognition algorithms, including ANNs, have been used widely to analyze biological sequences. Neural learning algorithms, such as back-propagation neural networks (BPNNs), self-organizing maps (SOMs), and recurrent neural networks (RNNs), have been used to analyze protein sequences (32). ANNs allow one to investigate complex, nonlinear relationships. Neural networks are therefore ideally suited for use in drug design (33, 34).

## Objectives

ANNs were used to make resistance phenotype predictions from biochemical and biophysical features of the YMDD sequence.

## Patients and Methods

### *Patient and samples*

Sera samples were collected from patients who were intended for surgery in various hospitals in Tehran-Iran (35). Patients who were infected with HBV who had not been treated with lamivudine and were negative for HCV and HIV markers were included. YMDD (wild-type pattern), YVDD, and YIDD mutant viral strains were distinguished by an in-house ACRS-PCR assay (36). Eleven samples were sequenced randomly to confirm the ACRS-PCR data.

### *Artificial Dataset*

The main purpose of this paper was to develop a new method based on artificial neural networks to simulate HBV drug resistance and compare its results with multiple regression models. There are several databases of HBV sequences. We used the DDBJ (http: //www.ddbj.nig.ac.jp/) and Expasy databases (http: //www.expasy.ch/) to develop our strategy, because they are used most often in the literature. To develop the model, HBV sequences from GenBank (http://www.ncbi.nlm.nih.gov/sites/entrez) and DDBJ were collected. The Expasy and HIV databases (http://www.hiv.lanl.gov/content/sequence/ENTROPY/entropy_one.html) were used to estimate biochemical parameters and entropy. *Table 1* shows the ranges of the parameters of the dataset. The input vector was represented by sequences with respect to HBV type. The primary database (DDBJ) that we used consisted of a set of oligo nucleotides from the literature. The oligos were compiled from published reports. The representational problem was addressed using different approaches, such as the definition and selection of physicochemical properties, the calculation of topological indices, and explicit vector-based representation of molecular connectivity. The exact number and type of descriptors that were used for a specific study were decided by an expert in the field.The encoding process required two subtasks to explicitly represent the relevant structural information in the molecules and to codify this structural information into a numerical representation.

### Statistical Criteria

The statistical and graphical criteria were adopted to select the desired optimal network model. The selection was performed based on statistical accuracy measures, such as root mean square error (RMSE), coefficient of determination (R2), and mean absolute error (MAE). In the training and testing stages, these parameters were used to determine the most promising optimal network. The ideal values of RMSE and MAE are zero; a value near zero indicates a better-performing model. Values for R2 range from 0 to 1; higher values indicate better model agreement.

### Network input, output, and preprocessing

After construction of the datasets that consisted of 700 amino acid sequences, they divided into two categories: resistant or sensitive to lamivudine. We assigned a value of 1 for resistance and 2 for sensitivity. Twenty-nine biochemical properties *(Table 1)*, Shannon entropy, and different domains of each gene were calculated using available software mentioned above. All of the governed parameters were then normalized to between 0.1 to 0.8 and sorted randomly to reinforce the performance of the procedure under random conditions.

### Model Development

The goal was to determine the relationship between chemiophysical features of mutant HBV YMDD and drug resistance. Construction of a training set that has patterns of a fixed length is difficult. A master list of chemiophysical features was compiled, including all features that appeared to be responsible for variations in contact point. Thus, the in put pattern that corresponds to a mutant has a pattern of features that leads to contact with the drug in a sensitive or resistant manner. The set of constructed patterns was then divided into a training set of 300 patterns and a validation

**Table 1.** The statistical parameters of dataset and the MLR equation coefficients

| Parameter | Statistics | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | Std Deviation | Variance | Skewness | Kurtosis | Minimum | Maximum | MLR Coefficient |
| **aa Number** | 118.1904 | 23.30669 | 603.5575 | -0.25747 | -1.60215 | 83.7 | 156.6 | -0.455 |
| **Molecular weight** | 13866.96 | 3108.255 | 10734724 | -0.26862 | -1.60774 | 9389.61 | 18367.92 | 0.005338 |
| **pI** | 6.327474 | 2.090067 | 4.853756 | -0.3488 | -1.46462 | 0.08316 | 8.361 | -0.20592 |
| **Ala** (A) | 3.819443 | 1.55822 | 2.697833 | 0.153344 | -0.584 | 0.9 | 7.83 | 0.028374 |
| **Arg** (R) | 7.018925 | 3.174779 | 11.19913 | -0.12186 | -1.4621 | 1.89 | 11.97 | 0.539712 |
| **Asn** (N) | 2.707171 | 0.969337 | 1.044014 | -0.00856 | -0.33279 | 0.9 | 5.85 | 0.083216 |
| **Asp** (D) | 5.155817 | 2.226123 | 5.506249 | 0.324161 | -0.56204 | 1.71 | 10.98 | 0.321206 |
| **Cys** (C) | 6.723824 | 0.896244 | 0.892504 | 0.97551 | 0.231891 | 5.49 | 9.36 | 0.746696 |
| **Gln** (Q) | 5.68506 | 2.091403 | 4.859963 | 0.913855 | 0.517726 | 2.43 | 12.15 | 0.123994 |
| **Glu** (E) | 7.225817 | 1.849778 | 3.801865 | 0.485804 | -0.54281 | 4.59 | 11.88 | 0.305088 |
| **Gly** (G) | 2.759881 | 1.082965 | 1.303124 | 0.940036 | 0.038038 | 1.17 | 5.49 | 0.515727 |
| **His** (H) | 3.064661 | 1.042359 | 1.207236 | -0.30858 | 0.691933 | 0 | 6.21 | -0.01709 |
| **Ile** (I) | 4.244702 | 0.903634 | 0.907283 | -0.3925 | -0.31292 | 1.71 | 6.75 | -1.19643 |
| **Leu** (L) | 10.03518 | 1.478487 | 2.428803 | 0.180419 | -0.42009 | 6.57 | 12.87 | -1.29542 |
| **Lys** (K) | 4.255817 | 2.2008 | 5.381689 | -0.4667 | -0.91863 | 0 | 7.56 | 0.738991 |
| **Met** (M) | 1.322032 | 0.775334 | 0.667935 | 0.656632 | -0.12146 | 0.54 | 4.32 | 0.571552 |
| **Phe** (F) | 2.290876 | 1.234493 | 1.693304 | -0.15179 | -0.23795 | 0 | 6.57 | -0.30452 |
| **Pro** (P) | 4.148247 | 1.055127 | 1.236992 | -0.05505 | -1.05318 | 1.71 | 6.48 | 0.122032 |
| **Ser** (S) | 4.255099 | 1.257226 | 1.756241 | 0.955589 | 1.028613 | 1.26 | 8.1 | 0.064222 |
| **Thr** (T) | 5.714104 | 2.360066 | 6.188786 | 1.191432 | 1.110611 | 0.9 | 11.97 | 0.168044 |
| **Trp** (W) | 0.684143 | 0.686557 | 0.523733 | 0.596039 | 0.114345 | 0 | 3.15 | 0.413734 |
| **Tyr** (Y) | 3.921634 | 2.081592 | 4.814474 | -0.20467 | -0.7231 | 0 | 7.56 | 0.053179 |
| **Val** (V) | 5.03749 | 1.606633 | 2.868077 | 0.964922 | 1.658503 | 2.25 | 10.98 | -0.74415 |
| **Asp + Glu** | 15.64064 | 2.045234 | 4.647758 | -0.76209 | -0.45523 | 10.8 | 18 | -0.24517 |
| **Arg + Lys** | 16.00279 | 8.950353 | 89.00981 | -0.22113 | -1.61159 | 3.6 | 27 | -0.56683 |
| **Sulfur** | 10.4522 | 2.286435 | 5.80865 | 0.85788 | 0.566699 | 6.3 | 16.2 | -0.50919 |
| **Ext. coef.** | 14222.67 | 9224.946 | 94500000 | 0.385209 | -0.66838 | 337.5 | 37606.5 | -5.9E-05 |
| **instab. indx** | 54.08089 | 9.106119 | 92.13489 | -0.42181 | -0.31483 | 26.037 | 71.946 | -0.00481 |
| **Aliph.** | 73.99043 | 7.536556 | 63.11075 | 0.412586 | 0.192556 | 59.976 | 95.904 | 0.360459 |
| | | | | | | | Constant | -25.486 |

set of 200 patterns. The training set included approximately 50% of the patterns with known resistance and 50% patterns without resistance values for a total of 300 training patterns. The validation set contained only patterns with known resistance and was used to test the prediction of the system. Finally, a network was trained with the patterns above.In the classical self-organizing feature map (SOFM) used here, all inputs were connected to all neurons. When a pattern is presented, the excitation of each unit is proportional to the dot product between the input vector and the weight vector. The unit with the weight vector that is closest to the input vector will have the largest excitation and will be declared the winner. The training involves changing the weights of the winner and its neighbors such that their weight vectors become more similar to the current input pattern. The training stops when the learning rate becomes zero. The experiments were performed with a learning rate of 0.6–0.9, and the learning rate was decreased linearly to zero over 10–50 training cycles

## Results

Using the collected dataset, a new model for predicting drug resistance in HBV was developed based on the ANN method. The results of the new MLP models are presented in *Table 2* for the training, validation, and testing steps, and the statistical results of this model are presented in *Table 3*. The input parameters of this model are in *Table 1*, and the output parameter was HBV drug susceptibility.The ANN model extracted the dominant phenomena of resistance in the HBV RT gene and simulated its chemiophysical processes. Based on the results of *Table 3*, the R2, RMSE, and MAE values of the MLP in the training stage were 0.8834, 0.07, and 0.09, respectively, and 0.8465, 0.160.04 in the testing stage; based on the total data, the values were 0.8549, 0.115, and 0.065, respectively. In the MLR model, the values in the training stage were 0.799, 0.159, and 0.214, respectively, *(Figure 1,2)* and 0.4225, 0.9, and 0.26 in the testing stage, respectively; based on the total dataset, the values were 0.6107, 0.5295, and 0.237, respectively. From *Table 3*, it is clear that the MLP model is superior to the MLR model in predicting HBV lamivudine resistance. Also, in *Table 3*, the error prediction of the MLP and MLR models is shown. The results show that ANN models can be used as alternative methods for predicting the outcome of HBV therapy. The test results of different networks with regard to predicting drug responses are shown in *Table 4*.

## Discussion

HBV drug resistance was predicted as a case study in this report. The proposed predictor showed vigorous clusterization of the predicted and observed drug responses. The current study was designed to find an algorithm that predicts drug resistance using chemiophysical information with artificially created neural networks. The applied properties for the model must be and rapid. After a pattern recognition model is constructed, the algorithm can be used to determine the repertoire of chemiophysical properties that is required for a drug response. This complex can be defined as the resistance pattern. This pattern can be used in a correlation analysis with known regulatory and biochemical molecular pathways to build a molecular model of drug resistance and response. Multifarious predictor models have been introduced for this purpose using different in vivo and in vitro architectures and parameters.

An MLP with one hidden layer and Tanh Axon as the transfer function can predict HBV drug resistance with an accuracy of 80% to 91%. Changes in the network structure, such as the addition of hidden layers, reductions in the threshold,

**Table 2.** Test results for predicting drug responses

| No. | Observed Output | MLP1 Prediction | | MLP2 Prediction | |
|---|---|---|---|---|---|
| | | Level | Error% | Level | Error% |
| **1** | 2 | 1.72 | 22 | 1.96 | 17 |
| **2** | 2 | 1.72 | 22 | 1.96 | 17 |
| **3** | 2 | 1.72 | 22 | 1.96 | 17 |
| **4** | 2 | 1.96 | 17 | 2.08 | 21 |
| **5** | 2 | 0.68 | 81 | 0.32 | 98.6 |
| **6** | 2 | 1.04 | 63 | 2.24 | 27 |
| **7** | 1 | 1.3 | 45 | 1 | 25 |
| **8** | 1 | 1.6 | 75 | 1 | 15 |
| **9** | 1 | 1.6 | 75 | 1 | 15 |
| **10** | 1 | 1.6 | 75 | 1 | 15 |
| **11** | 1 | 1.6 | 75 | 0.9 | 15 |
| **12** | 1 | 1.2 | 35 | 1.9 | 25 |
| **13** | 2 | 1.8 | 20.5 | 1.9 | 15 |
| **14** | 2 | 1.7 | 26 | 1.9 | 17 |
| **15** | 2 | 1.7 | 24 | 1.9 | 18 |
| **16** | 1 | 0.9 | 19.4 | 0.9 | 16 |
| **17** | 1 | 0.8 | 26 | 0.9 | 18 |
| **18** | 1 | 0.9 | 25 | 0.9 | 18 |

alterations in the transfer function, randomization of data, and crossvalidation, did not improve the results, whereas in some cases, results with higher errors were obtained. Several other networks were used, some of which exhibited better results: generalized feed forward, fuzzy logic network, recurrent neural network, time-lag recurrent network, and modular neural network. However, the results from the multilayer perceptron network were most integral to rationale of error. In this study, the decision over the optimal structure was made on the basis of their compatibility with other applications and training time. However, decreasing the threshold 10-fold resulted in improved output. Ignoring a single ill-conditioned exemplifier, the error in the response of the rectified network ranged from 0% to 12%. Generalized feed forward, modular neural network, and RBF/GRNN/PNN are acceptable models of HBV drug response levels when trained with a 100-fold-decreased threshold and 20000 epochs.

Based on the data in *Table 4*, the performance of the networks can be classified as follows: the resistance level was predicted best by the multilayer perceptron and modular neural network, and the susceptibility level was predicted best by the multilayer perceptron, generalized feed forward, and modular neural network within the calculated ranges of error. Ignoring the single ill-conditioned test sample, the prediction by multilayer perceptron for two levels may be considered and for the final judgment on the response levels, other competent networks as described above should be consulted. In order to extend the objectives of the present study, an intelligent and multidisciplinary program should be developed based on information from different applications in similar investigations. This program aids in the automatic design of expert neural network architectures for each application.

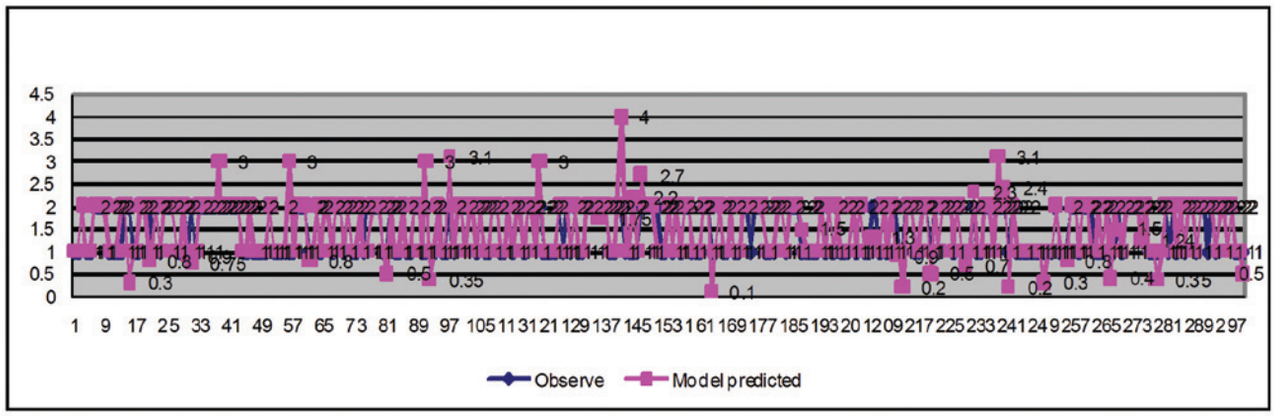


**Figure 1.** Comparison of the MLP model in the training stage with the observed values

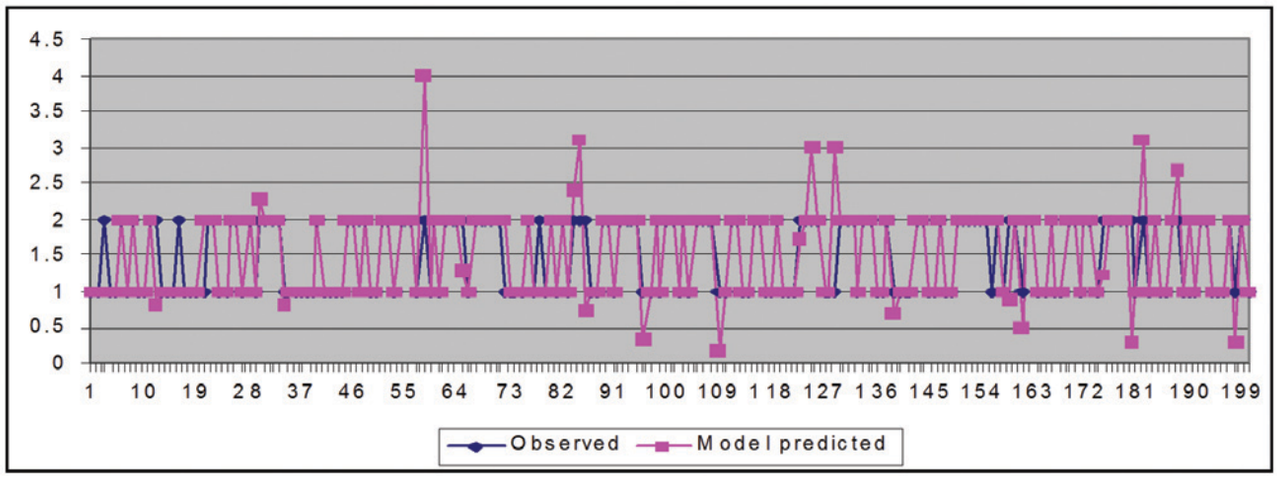


**Figure 2.** Comparison of the MLP model in the testing stage with observed values

**Table 3.** Comparison of the MLP model in the testing stage with observed values

| Model | Training | | | | Testing | | | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R2 | RMSE | MAE | Number of errors | R2 | RMSE | MAE | Number of errors | R2 | RMSE | MAE | Number of errors |
| MLP | 0.8834 | 0.07 | 0.09 | 25 | 0.8465 | 0.16 | 0.04 | 21 | 0.8549 | 0.115 | 0.065 | 46 |
| MLR | 0.799 | 0.159 | 0.214 | 37 | 0.4225 | 0.9 | 0.26 | 31 | 0.6107 | 0.5295 | 0.237 | 68 |

**Table 4.** Test results for different networks in prediction of oncogenicity

| No | GF | | MNN | | RBF/GRNN/PNN | |
|---|---|---|---|---|---|---|
| | Level | Eror. % | Level | Eror. % | Level | Eror. % |
| **1** | 2 | 17 | 2 | 15 | 2 | 19 |
| **2** | 2 | 17 | 2 | 15 | 2 | 21 |
| **3** | 2 | 19 | 2 | 15 | 2 | 21 |
| **4** | 2 | 41 | 2 | 19 | 2 | 21 |
| **5** | 1 | 99 | 1 | 87 | 1 | 35 |
| **6** | 1 | 89 | 1 | 77 | 2 | 19 |
| **7** | 1 | 15 | 1 | 15 | 1 | 35 |
| **8** | 1 | 55 | 1 | 16 | 1 | 35 |
| **9** | 1 | 65 | 1 | 16 | 1 | 35 |
| **10** | 1 | 65 | 1 | 16 | 1 | 35 |
| **11** | 1 | 25 | 1 | 15 | 1 | 35 |
| **12** | 2 | 15 | 1 | 35 | 1 | 45 |
| **13** | 2 | 16 | 2 | 15 | 2 | 30 |
| **14** | 2 | 15 | 2 | 16 | 2 | 24 |
| **15** | 2 | 15 | 2 | 16 | 2 | 31 |
| **16** | 2 | 15 | 2 | 16 | 2 | 37 |
| **17** | 2 | 16 | 2 | 15 | 2 | 26 |
| **18** | 2 | 15 | 2 | 16 | 2 | 33 |

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