ON THE EFFECT OF FALSE POSITIVES IN GROUP AND INDIVIDUAL TESTING OF BLOOD FOR THE PRESENCE OF HIV

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Abstract

In this thesis we develop a model which allows us to evaluate Modified Dorfman's and Modified Sterrett's group testing procedures in the presence of false positive classification error that can be used for screening blood for HIV. Performance measures derived are the expected number of tests per sample and the corresponding coefficient of variation. The procedures differ from the original ones by the fact that groups and samples are retested certain number of times before they are classified as positive. Modified Individual testing procedure and the procedure currently used by Canadian Red Cross are also evaluated and all four testing strategies are compared. Numerical analysis illustrates that group testing is more efficient than alternatives.

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Chapter 1
INTRODUCTION

Minimizing risk of deadly infections like HIV, Hepatitis-B and HTLV (human T-cell lymphotropic virus), from blood and blood products is a major preoccupation of the Canadian Red Cross. This agency is solely responsible for collection, testing and distribution of blood and its products in Canada. So important is the question of safety that a Commission of Inquiry, headed by Honourable Mr. Justice Horace Kreaver, is presently examining all facets of the Canadian Blood System in an attempt to make it safer. Whereas the risk of infection cannot be eliminated entirely (due to the limitations of the existing testing technology) Justice Kreaver in his interim report [4] strongly recommends that the controllable part of the risk be reduced as much as possible. In Recommendation 8 of his report, he underscores the need for research which includes "... evaluation of strategies, procedures and policies aimed at improving safety and efficiency of the blood

system" [4, Vol.2, Annex 1, page 11]. Responding to this call to arms, we present testing strategies suitable for screening blood samples for presence of HIV antibodies which reduce both testing cost and risk of testing errors. We also provide a comparative evaluation of some existing protocols, including the one currently used by Canadian Red Cross.

The testing strategies we propose involve the group testing approach. Unlike individual testing, this approach results in pooling samples together and testing them as a group. If a group is classified as negative, then all of its members are classified as negative in a single test. Otherwise, a specified procedure is employed to identify samples from the group which should be classified as positive. Some such procedures are shown schematically in Figure 1.

The standard test for HIV antibodies is called *ELISA* which stands for Enzyme Linked Immuno Sorbent Assay. A more precise but expensive confirmatory test, called the Western Blot, is performed on samples which are found to be positive on initial screening with ELISA (see schematic in Figure 2). Since both tests detect antibodies, neither can detect infected samples during the "window period" – a period of up to 12 weeks during which the infected individual does not develop sufficient quantities of the antibodies to be detected by these tests. To combat this problem, Canadian Red Cross administers a questionnaire (Figure 3) as well as a personal interview to

all donors to filter out individuals who might belong to the "high risk" category. The donors can also anonymously indicate whether for some reason they consider the use of their blood to be risky. These steps have two additional benefits. First, they reduce the effective prevalence rate among the eligible pool of donors, thereby minimizing risk. Second, they also create a situation favourable for implementing group testing since group testing strategies are most effective when prevalence rate is small. The "Window Period" problem might be avoided if PCR (Polymerase Chain Reaction) assay was used instead of ELISA, since this method is based on the detection of antigens (predecessors of antibodies) rather than antibodies. The use of PCR for HIV testing is currently being researched [14].

While much work has been reported in the medical literature on the feasibility of testing in groups using ELISA technique [1,3,6], and in the statistical literature on group testing strategies and their benefits (see Chapter 2 for a summary of relevant literature), Canadian Red Cross and other agencies around the world are not currently using a group testing approach. This fact is particularly unfortunate in light of the fact that the group testing strategies, when feasible, are particularly beneficial when prevalence rates are low. We believe that part of the reason for this lies in the need for testing strategies to be user friendly. The procedure must be

made simple to avoid a possibility of human error, for example, during labelling and handling. Furthermore, the maximum number of tests to be performed on each sample must be small.

The accuracy of any testing procedure can be described by two measures: *sensitivity* and *specificity*. The sensitivity of the test is the proportion of truly positive samples which are identified as positive by the test. Specificity, on the other hand, is the proportion of negative tests which are truly negative. When a test fails to return a positive result for an infected sample, the error is known as a *false negative*. On the other hand, when the test returns a positive result for a sample which is not infected, the error is known as a *false positive*. In ELISA and other tests, the *threshold* which separates a negative test result from a positive one, can be set so as to achieve some desired level of sensitivity. Unfortunately, just as a more sensitive burglar alarm system is more likely to be triggered inadvertently, greater sensitivity is achieved at the cost of loss of specificity or greater incidence of false positives. Given a high cost of a false negative, Canadian Red Cross takes a conservative approach and rejects all blood samples that show initial positive results, even when subsequent confirmatory testing produces negative outcomes [11]. This increases the incidence of false positives and decreases the effective "yield" of good

blood from the samples collected from donors. As the Canadian supply of blood is dependent upon volunteer donors only, yield losses can also be a serious problem.

The test parameter which is used by the Canadian Red Cross staff for negative and positive blood classification is a quantity called optical density (OD). OD is determined by the number of antibodies in a blood sample. OD readings which exceed some threshold value result in blood samples being classified as infected. A good description of the chemistry of the ELISA testing procedure is given by Wein and Zenios [15]. The cut-off value is set equal to the mean of negative controls used in the testing procedure plus 0.25 [6]. Clearly the cut-off is kept small in an attempt to avoid false negative classifications. It is nevertheless possible to make this threshold value even smaller so that test sensitivity is very close to 1 and the probability of a false negative outcome is almost zero. In this case we will inevitably observe more positive classifications or, to be more specific, more false positive classifications. Therefore, this situation would also require a mechanism for retesting samples which produce a positive outcome on the first test so that the final classification does not include a large number of false positive samples. We employ such a retesting scheme in our analysis. It first assumes that probability of a false negative outcome is zero. Then the number of retests necessary

to classify a sample or a group of samples as positive is obtained so that the probability that an individual sample or a group is classified accurately is very close to 1.

We develop expressions for two performance measures which we have chosen to be the expected number of tests per sample and the corresponding coefficient of variation. These performance measures were chosen since they reflect two features important for blood screening application: cost in terms of the number of tests and variability of outcomes. Next we compare modifications of two procedures originally proposed by Dorfman and Sterrett, and the method for screening blood for HIV currently used by Canadian Red Cross, in terms of these measures. The modified procedures include a retesting component as explained above.

Our numerical analysis illustrate that Modified Dorfman's (MD) and Sterrett's (MS) procedures perform much better than the alternatives: Modified Individual (MI) testing and the procedure currently used by Canadian Red Cross (CP). MS is slightly better than MD but, due to the fact that MS is harder to implement, we recommend that MD be used unless even small savings are deemed important.

Even though our discussion is concerned specifically with the application of screening blood for HIV, it should be noted that the problem of complete classification of items which form a large population also arises in a variety of

business applications. In a number of such applications it is possible to test groups of items in a single test at costs comparable to those necessary for testing an individual item. For example, testing containers for leakage consists of putting containers filled with spreadable material in a device which can register the presence of that material outside of the containers. Connecting light bulbs or other electric devices in series produces another example of group testing which leads to identification of defective components. For certain cost structures, and low rates of incidence of defectives, a group testing strategy turns out to be a cost effective alternative.

Next chapter gives review of relevant literature. Chapter 3 contains the mathematical description of the model as well as derivations of main results. The blood screening procedure currently used by Canadian Red Cross is discussed in Chapter 4. Our numerical analysis and conclusions can be found in Chapter 5.

Chapter 2

REVIEW OF RELEVANT LITERATURE

The idea of group testing was initially put forward by R. Dorfman in 1943 [5]. He considered an application of screening men called up for induction in the army for syphilitic antigen and proposed pooling blood samples together and testing them as a group. If the group test was found positive, samples were then tested individually. On the other hand, if the group test was negative, group members were classified as negative in a single test. The goal of Dorfman's analysis was to determine under what conditions and for what group sizes the grouping technique would require a smaller number of tests on average than the individual testing. Defining p as prevalence rate, n as sample size and N as population size, Dorfman derived an expression for the expected number of tests:

$$E(T_N) = \frac{N}{n} + N(1-(1-p)^n). \qquad (1)$$

Here T_N is a random variable which denotes the total number of tests needed to classify all items of a population of size N. The performance measure used by Dorfman is the expected relative cost, C, which is the expected total number of tests required to classify each blood sample:

$$C = \frac{n+1}{n} - (1-p)^n. \qquad (2)$$

Optimal group sizes are found by locating the minimum of C with respect to n for fixed values of p. Dorfman concludes that his group testing procedure is more efficient than individual testing if prevalence rate is sufficiently small. Smaller p values are associated with larger savings. In this thesis we discuss a modified version of Dorfman's procedure (MD) which explicitly models the presence of false positive outcomes.

The group testing procedure described by Dorfman has been generalized in several ways by different researchers. For instance, Sterrett [13] proposes that individual testing be performed on a defective group only until first defective sample is located. Then the remaining samples are tested as a group and this procedure continues until all samples of the original group are classified. Employing the same notation as

Dorfman, but denoting group size by k, Sterrett derived the expression for the total expected number of tests necessary to classify all samples in the population if his procedure is applied, which is denoted by $E(N,k,p)$. Conditioning on the number of defective samples in the group, and denoting the probability that a group of size k has exactly i defective members by $Pr_k(i)$ and the expected number of tests needed to isolate these i members by $E_k(i)$, the expected total number of tests can be written as follows:

$$E(N,k,p) = \frac{N}{k}\sum_{i=0}^{k} Pr_k(i)E_k(i). \qquad (3)$$

Using a recursive relationship among $E_k(i)$'s Sterrett derives the general formula:

$$E_k(i) = \frac{i}{i+1}k+i+1+\frac{i}{i+1}-\frac{2i}{k}. \qquad (4)$$

Using the fact that $Pr_k(i)\approx 0$ for i close to k, and assuming that p is small, a simple approximation for $E(N,k,p)$ can be obtained. Let $E'(N,k,p)$ denote this approximate value. Optimal group sizes are determined by minimizing $E'(N,k,p)/N$ with respect to k for fixed values of p. Sterrett's numerical analysis shows that his procedure is more efficient than Dorfman's by about 6% if prevalence rate is small. We develop a new model which modifies Sterrett's procedure to include the presence of false positives. Also, we evaluate both the

expected value and the coefficient of variation of the total number of tests needed to classify each sample. This procedure is called the Modified Sterrett's procedure (MS). Figure 1 shows a schematic description of Dorfman's and Sterrett's procedures.

One way to extend Dorfman's analysis to take into account testing errors was proposed by Graff and Roeloffs [7]. They have used retesting in their method to reduce the possibility of wrong classification. Four parameters which correspond to the number of retests were defined as follows: "If a group of size $x>1$ yields $r1$ defective readings before it yields $r2$ good readings, it is classified as defective and each item is tested individually. Otherwise, it is classified as good. A single item is classified as defective if it yields $s1$ defective readings before it yields $s2$ good readings. Otherwise, it is classified as good."

The authors introduce two costs of misclassification and derive the expression for the expected cost of classifying all items in the group of size x, $C(x,r1,s1,r2,s2)$. The cost of a single test (group or individual) is treated as the unit of cost. The expected cost per sample is minimized over the values of the parameters. The optimal values are found as relative minima by comparing the values of the considered expression at the points adjacent to some arbitrarily chosen initial point.

In this thesis we take a different approach and use a different model to incorporate testing errors in the analysis of the group testing procedures because the true costs of misclassification are difficult to estimate. False negatives may cost lives of people who receive blood transfusions and may result in lawsuits. False positive classification, on the other hand, leads to retesting using expensive Western Blot test, reduces yield of good blood from samples collected and leads to further testing due to the fact that healthy people whose blood is rejected seek confirmation. It may also cause anxiety and emotional trauma in the person whose blood is rejected [2]. Whereas it is difficult to evaluate cost of potential loss of life and emotional trauma in dollar terms, managers are often comfortable with the idea of an acceptable level of incorrect classification, such as we use in our model.

Sobel and Groll [12] have proposed yet another generalization of Dorfman's procedure. They suggested that once a positive group is obtained, it may be more efficient to take a subgroup from it and treat it as the new group rather than do individual testing. In their analysis a population of N unclassified items is assumed to be binomial with respect to the characteristic of interest, i.e., items are either good or defective. At each point in time the population is divided into three sets: defective (at least one item in the set is

defective), binomial (a set of unclassified items which are treated as if they were never tested) and a set of classified items. Sizes of these sets are m, $n-m$ and $N-n$, respectively. Sobel and Groll proposed to take a group of samples of size x from a defective set and test it. If the result of this test is positive the rest of the defective set, i.e., the set of $m-x$ samples, is joined with the binomial members and a new group of items is drawn from the defective group of size x. Otherwise, the remaining portion of a defective set becomes the source of the next subgroup to be tested, i.e., it becomes the next defective set. This process continues until all N samples are classified. For various prevalence rates and several N values optimal group sizes are found for each of the situations described above. Group sizes are optimal in the sense that they minimize the total expected number of tests necessary to classify all items. The recursive formulae which define the above procedure are:

$$H_1(n) = 1 + \min_{1 \le x \le n} (g^x H_1(n-x) + (1-g^x) G_1(x,n)), \tag{5}$$

$$G_1(m,n) = 1 + \min_{1 \le x \le m-1} \left(\frac{g^x - g^m}{1-g^m} G_1(m-x,n-x) + \frac{1-g^x}{1-g^m} G_1(x,m) \right),$$

with boundary conditions $H_1(0)=0$, $G_1(1,n) = H_1(n-1)$. The parameter g denotes the probability of a sample being

uninfected, $G_1(m,n)$ denotes the expected number of tests remaining to be done when defective and binomial sets are of sizes m and $n-m$, respectively, and $H_1(n)$ denotes the same quantity when $m=0$. One of the important differences between this procedure and the protocols previously discussed is that it allows unclassified subsets of a defective set to return to the binomial population while other procedures concentrate solely on the defective set until all of its items are classified. This procedure also requires the knowledge of the population size in order to choose initial group and this information might not always be available. It is obvious that Sobel and Groll's procedure is more difficult to apply than procedures previously discussed due to the fact that the test group size is not fixed, even when prevalence rate and test accuracy do not change. In an attempt to avoid this difficulty Sobel and Groll consider a simplified version of their protocol which involves halving the defective set at each decision stage until every sample is classified. Sobel and Groll concluded that their procedure is more efficient than Dorfman's and Sterrett's and that the halving procedure is nearly as good as their more complicated method (Table 1). Procedures discussed above can also be extended to include false positive and false negative outcomes but, since they are quite complicated to implement even without such considerations and user-friendliness is important in blood

screening and many other applications, we chose not to do so.

A modified version of Sobel and Groll's halving procedure was proposed by Litvak, Tu and Pagano [10]. They recommended splitting positive groups in various subgroups of almost equal sizes rather than concentrating on a single subgroup of a positive group. These subgroups are then tested and split further if outcomes are positive. Whenever a group produces a negative result it is retested at most *r-1* times. If all *r-1* results are negative the group is classified as negative. Otherwise, it is classified as positive. Each procedure of

TABLE 1 * EXPECTED NUMBER OF TESTS PER ITEM
FOR VARIOUS GROUP TESTING PROCEDURES

PREVALENCE RATE	DORFMAN'S	STERRETT'S	SOBEL and GROLL'S	HALVING
0.01	0.20	0.14	0.13	0.13
0.02	0.27	0.22	0.17	0.17
0.05	0.43	0.35	0.30	0.30
0.10	0.59	0.51	0.48	0.50
0.25	0.91	0.84	0.83	1.03

*This table is from Sobel and Groll [2,page 1227].

this type is specified by the number of negative tests necessary to declare groups or samples negative, the initial group size and a vector of divisors. The authors compared

several such procedures with respect to the expected number of tests, and the false positive and false negative predictive values. The latter are denoted by $FPPV(T)$ and $FNPV(T)$ and defined as follows:

$$FNPV(T) = \frac{p(1-S_e(T))}{p(1-S_e(T))+(1-p)S_p(T)}, \qquad FPPV(T) = \frac{(1-p)(1-S_p(T))}{(1-p)(1-S_p(T))+p\,S_e(T)}. \tag{7}$$

Here $S_p(T)$ and $S_e(T)$ denote specificity and sensitivity of a procedure T used to classify all samples. These quantities depend on T, prevalence rate and the specificity and sensitivity of the test kit used. All procedures discussed were applied to a sample of size 15, with sensitivity and specificity equal to 0.995, and r=2, and a comparative evaluation was performed for various values of the prevalence rate. Numerical analysis has shown that for small prevalence rates Dorfman's procedure requires smaller expected number of tests but results in higher false positive and false negative predictive values. The authors, therefore, conclude that their procedure should be used since the quality of results is an important issue, especially in a blood screening application.

Wein and Zenios [15] consider the problem of classification from a different angle. In an attempt to capture the dilution effect (failure of a test to detect an infection due to the

positive blood sample being mixed with a large number of negative samples) Wein and Zenios develop a generalized linear model which connects optical density levels (OD, or the ELISA test outcome) with the antibody concentration in the grouped sample and then use it in their dynamic programming algorithm to produce a group testing policy for which expected cost due to false negatives, false positives and testing is minimized. It should also be noted that, unlike previous studies, these authors allow three possibilities at each stage of testing: to declare all group members negative, require further testing as prescribed by their model, and to declare all group members positive. Using a large data set Wein and Zenios have investigated the distribution of the logit transformation of normalized OD readings ($x \rightarrow \ln(x/(1-x))$) and determined that it is normally distributed for both positive and negative individuals. Values of suitable thresholds (one for each of the three decision outcomes) could then be found from these results (see Figure 4). Wein and Zenios conclude that the policy they consider, which also uses different threshold values at different stages of testing is a good way to control false classifications.

Johnson, Kotz and Wu [9] survey group testing strategies for their applications in quality control. They analyze Dorfman's, Sterrett's and some other procedures by replacing

simple probabilities involving only prevalence rate and group size with probabilities which allow the probabilities of false negative and false positive outcomes. The authors have also applied Graff and Roeloff's approach [7] to what they call hierarchical screening which is the same procedure that Litvak, Tu and Pagano [10] have proposed.

Other researchers have considered various other extensions of group testing method (see Johnson, Kotz and Wu [9] for additional references). For example, Hwang [8] discusses several group testing procedures under the assumption that different non-conforming rates may be present. Misclassification errors are set to zero. This author also defines a general class of two-stage procedures which is described by the following two properties: a) once optimal group size is determined it is used until all items of a large population are classified, b) once a group is classified as positive it is dealt with until all of its members are classified. Notice that these principles are present in all of the procedures we have discussed previously except for the procedure of Sobel and Groll.

Chapter 3

THE MODEL

3.1 ASSUMPTIONS

As mentioned earlier it is our intent to incorporate the classification errors in the model for evaluation of group testing strategies. It is clear from Figure 4 that a threshold can be chosen to control either the risk of false negatives or the risk of false positives. Rather than dealing with two misclassification errors by explicitly incorporating both of them in the model, we propose to eliminate the possibility of a false negative outcome (which is by far much more undesirable) by setting the threshold in such a way that samples classified as negative will have very low OD reading that can only correspond to an uninfected blood. Under the system described above the only way we can get a false negative sample is if the blood comes from an individual in the "window period". This phenomenon is very rare in the

general population and it occurs even more seldom in the population of donors due to several layers of screening procedures that potential donors must undergo. It is also important to add that PCR, a test currently being considered as a substitute of ELISA, does not have the drawback of not detecting infection in a sample from a person in the "window period". These reasons lead us to the first assumption for our model:

(1) $pf_- = Probability\ (Sample\ produces\ a\ negative\ result | Sample\ is\ infected) = 0$.

Setting the threshold to an extreme (small) value will result in larger number of false positive outcomes. This problem can be dealt with by retesting samples and groups which produce positive reading on the first test. Once the number of retests is set to a specific value, say r, the following rule will be followed: if a sample or group of samples produces a positive outcome on the first test then retest it $r-1$ times or until the first, if any, negative reading is obtained. If all $r-1$ tests are positive classification should be positive. Otherwise, classification is negative. It was suggested by Litvak, Tu and Pagano [10, page 141] that usually the retesting of positives leads to higher number of false negatives. When both of the testing errors are present this claim is valid but if the situation corresponds to the case when $pf_-=0$, as it is under our model,

retesting of positives will lead to the reduction of false positive classifications only.

In all of the derivations for our model the following was assumed:

(2) Blood samples are statistically independent.

3.2 NOTATION AND ABBREVIATIONS

Before any of the results can be proved it is necessary to introduce the notation used throughout the thesis.

p -prevalence rate;

n -sample size;

pf_+ -probability of a false positive outcome (Probability (Sample produces a positive result|Sample is uninfected));

α -minimum tolerable probability of a sample or a group of samples being infected given that this sample or a group is classified as positive;

$r1$ -number of consecutive positive test outcomes necessary to classify a sample as positive;

r -number of consecutive positive test outcomes necessary to classify a group of samples as positive;

$pi_+(n)$ -probability of getting a positive result on the i-th test given that all previous $i-1$ test results

were positive when a group of size n is considered;

$pi(n)$ -probability of a group of size n being infected given that $i-1$ consecutive tests on this group were positive.

The set of the random variables employed in our derivations is defined next:

X_i -number of tests necessary to classify a sample (i-th sample) as positive given that this sample is eventually classified as positive;

Z_i -number of tests necessary to classify a sample (i-th sample) as negative given that this sample is eventually classified as negative;

Y -number of samples classified at the moment we stop individual testing on samples from the group classified as positive during Sterrett's procedure;

V_i -number of tests necessary to classify a sample (i-th sample);

T_n -number of tests necessary to classify all samples in a group of size n;

T_n^+ -number of tests necessary to classify all samples in a group of size n which has been classified as positive.

Due to the fact that formulas derived in this thesis are sometimes lengthy, the following abbreviations are used to simplify them:

(1) $F = 1-pf_+$; (2) $D = 1+pf_+$;

(3) $B = (pf_+)^{r1}$; (4) $B_n = (pf_+)^r$;

(5) $C = p+(pf_+)^{r1}(1-p) = 1-(1-p)(1-B)$;

(6) $A = 1-(pf_+)^{r1}(r1+1)+r1(pf_+)^{r1+1}$;

(7) $A_n = 1-(pf_+)^r(r+1)+r(pf_+)^{r+1}$;

(8) $H = \frac{[DA-r1(r1+1)BF^2](1-p)}{F^2}$;

(9) $H_n = \frac{[DA_n-r(r+1)B_nF^2](1-p)^n}{F^2}$;

(10) $P = r1(1-(1-C)^n)+\frac{A(1-p)\{1+[C(1-n)-1](1-C)^{n-1}\}}{FC}$;

(11) $S_1 = \frac{H}{1-C}+\frac{2r1A(1-p)}{F(1-C)}$;

(12) $S_2 = 1-(1-C)^n(n+1)+n(1-C)^{n+1}$;

(13) $S_3 = [\frac{A(1-p)}{F(1-C)}]^2$;

(14) $S = Cr1(1-r1) + H(1-C)^{n-1}n+n(n-1)S_3(1-C)^n+$

$$[1-(1-C)^n][2S_3-S_1+(r1)^2]+\frac{S_2(S_1-3S_3)}{C}+$$

$$\frac{S_3[(2-C)S_2-n(n+1)C^2(1-C)^n]}{C^2} .$$

Using the notation presented above important results are derived in the next section.

3.3 GENERAL RESULTS

Results presented in this section are going to be useful in the derivations of expressions of the expected number of tests and the variance of the total number of tests for individual testing strategy and group testing procedures. Since retesting is performed if the first test is positive it is necessary to find the formula for the probability of a positive test outcome at every stage of retesting. This is done in Result 1.

RESULT 1:

$$pi_+(n) = \frac{1-(1-p)^n+(pf_+)^i(1-p)^n}{1-(1-p)^n+(pf_+)^{i-1}(1-p)^n} .$$

Proof: We start with the formula which connects $pi_+(n)$ and $pi(n)$:

$$pi_+(n) = pi(n) + (pf_+)(1-pi(n)) . \qquad (7)$$

When a group of samples is tested for the first time the following is true: $p1(n)=1-(1-p)^n$ and $p1_+(n)=1-(1-p)^n+(pf_+)(1-p)^n$. For the second test, using the formula for the

conditional probabilities and the fact that false negative outcomes can not occur, it easily follows that

$$p2(n) = \frac{p1(n)}{p1(n)+(pf_+)(1-p1(n))}.$$

Combining the above expression with (7) yields:

$$p2_+(n) = \frac{p1(n)+(pf_+)^2(1-p1(n))}{p1(n)+(pf_+)(1-p1(n))}.$$

For the third test the probabilities are derived in a similar manner:

p3(n)=Pr(Sample is infected| Two test results were positive)=

$$= \frac{p1(n)}{Pr(Two\ test\ results\ are\ positive)} =$$

$$= \frac{p1(n)}{Pr(Second\ test\ is\ positive/First\ test\ is\ positive)}$$

$$\frac{1}{Pr(First\ test\ is\ positive)} = \frac{p1(n)}{p2_+(n)\,p1_+(n)} =$$

$$= \frac{p1(n)}{p1(n) + (pf_+)^2(1 - p1(n))}$$

and, using (7),

$$p3_{+}(n) = \frac{p1(n) + (pf_{+})^{3}(1 - p1(n))}{p1(n) + (pf_{+})^{2}(1 - p1(n))} .$$

Recognizing the pattern demonstrated above, it becomes clear that the following general formula is true:

$$pi(n) = \frac{p1(n)}{p1(n) + (pf_{+})^{i-1}(1 - p1(n))} . \qquad (8)$$

Formulas (7) and (8) can be combined to obtain the general expression for $pi_{+}(n)$. The final result is obtained by replacing $p1(n)$ by $1-(1-p)^{n}$ and simplifying.■

A few comments can be made with regard to Result 1. As expected, $pi(n)$ approaches 1 as i increases. Secondly, smaller values of pf_{+} will result in $pi(n)$ getting closer to 1 faster. This is reasonable since when the probability of a false positive classification is close to 0 then even one positive test outcome indicates that a group or a sample is probably infected. It should also be noted that, as i increases, $pi(n)$ increases from $1-(1-p)^{n}$ to the value close to one.

As expectations and variances are derived in later sections a few expressions come up several times. They are simplified and presented in Results 2, 3, 4 and 5. Result 2 simplifies the probability of getting positive test outcomes r times or

classifying a group of size n as positive.

RESULT 2:

$$\prod_{i=1}^{r} pi_{+}(n) = 1-(1-p)^{n}+(pf_{+})^{r}(1-p)^{n} .$$

Proof: When $pi_{+}(n)$'s are multiplied a lot of cancelations occur since numerators and denominators of each pair $[pi_{+}(n)][p(i-1)_{+}(n)]$ are the same. In the end we will be left with the numerator of the last term in the product, namely, $pr_{+}(n)$. ■

Result 3 and 4 deal with the part of expectations which corresponds to the negative classification of a group of size n.

RESULT 3:

$$\sum_{i=2}^{r} i[\prod_{j=1}^{i-1} pj_{+}(n)](1-pi_{+}(n))+1-pl_{+}(n) = \frac{(1-p)^{n}[1-(r+1)(pf_{+})^{r}+r(pf_{+})^{r+1}]}{1-pf_{+}} .$$

Proof: We start by applying Results 1 and 2 to the left hand side (LHS) of the expression above and simplifying:

$$LHS = \sum_{i=1}^{r} i(pf_{+})^{i-1}(1-p)^{n}(1-pf_{+}) .$$

Result 3 follows once expression $(1-p)^{n}(1-pf_{+})$ is taken out of the summation and the following formula is applied:

$$\sum_{i=1}^{k} iq^{i-1} = \frac{1-(k+1)q^k+kq^{k+1}}{(1-q)^2} \qquad (0\le q\le 1). \qquad (9)$$

Formula (9) can be verified by first noting that $iq^{i-1}= \partial(q^i)/\partial(i)$ and interchanging the order of summing and taking derivative. Final result is obtained after geometric series formula is applied and the expression is simplified.■

RESULT 4:

$$\sum_{i=2}^{r} i^2[\prod_{j=1}^{i-1} pj_+(n)](1-pi_+(n)) + 1 - pl_+(n) =$$

$$= \frac{(1-p)^n\{(1+pf_+)[1-(r+1)(pf_+)^r+r(pf_+)^{r+1}]-r(r+1)(pf_+)^r(1-pf_+)^2\}}{(1-pf_+)^2}.$$

Proof: We start by applying Results 1 and 2 to the left hand side (LHS) of the expression above and simplifying:

$$LHS = \sum_{i=1}^{r} i^2(pf_+)^{i-1}(1-p)^n(1-pf_+) .$$

Result 4 follows once expression $(1-p)^n(1-pf_+)$ is taken out of the summation and the following formula is applied:

$$\sum_{i=1}^{k} i^2q^{i-1} = \frac{(1+q)[1-(k+1)q^k+kq^{k+1}]-k(k+1)q^k(1-q)^2}{(1-q)^3} \qquad (0\le q\le 1). \quad (10)$$

Formula (10) can be verified as follows:

$$\sum_{i=1}^{k} i^2 q^{i-1} = \frac{1}{q}\sum_{i=1}^{k} i^2 q^i = \frac{1}{q}\sum_{i=1}^{k} [q^2(\frac{\partial^2(q^i)}{\partial i^2} + iq^{i-2})] = q(\sum_{i=1}^{k} \frac{\partial^2(q^i)}{\partial i^2} + \frac{1}{q}\sum_{i=1}^{k} iq^{i-1}) \ .$$

First summation is evaluated by interchanging the order of the operations of summing and taking second derivative and applying the geometric series formula, while second sum is obtained from formula (9). Algebraic simplification of the resulting expression leads to the final result.■

RESULT 5:

$$\sum_{i=2}^{r} [\prod_{j=1}^{i-1} pj_+(n)](1-pi_+(n)) = (1-p)^n[pf_+ - (pf_+)^r] \ .$$

Proof: We start by applying Results 1 and 2 to the left hand side (LHS) of the expression above and simplifying:

$$LHS = (1-p)^n(1-pf_+)[\sum_{i=1}^{r} (pf_+)^{i-1} - 1] \ .$$

The final result can be obtained by applying the geometric series formula to the summation in the expression above and simplifying.■

RESULT 6:

$$r = \lceil \frac{\ln\{[1-(1-p)^n](1-\alpha)\} - \ln\{(1-p)^n\alpha\}}{\ln(pf_+)} \rceil \ .$$

Proof: As defined in section 3.2 r is the number of retests

or the number of times a sample or a group has to produce a positive result in order to be classified as positive. The criteria for selecting the number of retests is the following: a group or a sample has to be classified as positive in such number of tests that the probability of an infection in the considered blood given that this group or a sample is classified as positive is at least α(some number, usually, close to 1). The probability, mentioned above, is equal to $p(r+1)(n)$. Therefore, r should be chosen in such a way that: $p(r+1)(n) \geq \alpha$. We apply formula (8) to the left hand side of this inequality and use the fact that $p1(n)=1-(1-p)^n$ to arrive to the following expression:

$$\frac{1-(1-p)^n}{1-(1-p)^n+(pf_+)^r(1-p)^n} \geq \alpha .$$

Solving this inequality for r yields:

$$r \geq \frac{\ln\{[1-(1-p)^n](1-\alpha)\}-\ln\{(1-p)^n\alpha\}}{\ln(pf_+)} .$$

Since r is the number of retests it has to be a positive integer and should be as small as possible. This suggests that the ceiling of the right hand side of the above expression will serve as an appropriate r.■

It is expected to observe the decrease in the value of r when n(the group size) is increased since, as n gets larger,

the probability of a grouped sample containing an infected blood increases and, therefore less retests should be required to classify this group as positive. It can be easily verified that this is indeed the case by taking derivative of the expression for r with respect to n and observing that it is always negative.

In section 3.2 several random variables were defined, namely X_i, Y, Z_i and V_i. These variables are important because for MS it is possible to express the total number of tests needed to classify all considered samples in terms of these variables. This will be done in section 3.6. Next some useful results concerning these variables are derived.

RESULT 7:

$$E(X_i)=r1 \qquad E(X_i^2)=(r1)^2 \ .$$

Proof: First we have to recall that $r1$ is the number of times a sample must produce a positive result to be classified as positive. Therefore, a sample can be classified as positive in $r1$ tests only. This implies that $Pr(X_i=r1)=1$ and, consequently, result 7 follows.■

RESULT 8:

$$Pr(Y=k) = \begin{cases} C(1-C)^{k-1}, & k=1,2,\ldots,n-1. \\ (1-C)^{n-1}, & k=n. \end{cases}$$

Proof: In section 3.2 Y was defined as the random variable which denotes the number of blood samples classified at the

moment we stop individual testing during MS. According to the definition of Sterrett's procedure individual testing is terminated when one of the samples is classified as positive. During this process of testing individual samples one after another the following fact is true: when each sample is tested individually for the first time the probability of it being infected varies from sample to sample, because this probability is being updated as new information becomes available. By the new information we mean such facts as the group containing all of the samples being classified as positive and certain number of samples from this group already being classified as negative. The modelling of this updated probability is not an easy task and it seems that it does not change in a systematic way. This lead us to the decision to approximate that probability by prevalence rate, p, in all of our derivations. In the light of the above discussion the following expression can be written for $k=1,2,\ldots,n-1$:

$Pr(Y=k)$ = $Pr(k$-1 *samples are classified as negative and one is classified as positive*$)$ = $[(1-p1_{+}(1))+$

$$+\sum_{i=2}^{r1}\left(\prod_{j=1}^{i-1} pj_{+}(1)\right)(1-pi_{+}(1))]^{k-1}\prod_{i=1}^{r1} pi_{+}(1)\ .$$

This expression can be simplified by applying Result 5 to the

first term in the product and Result 2 to the second. Further algebraic simplification leads to the expression which can be written as the following using C, defined in section 3.2:

$$Pr(Y=k)=C(1-C)^{k-1},\ k=1,2,\ldots,n-1.$$

Expression for $Pr(Y=n)$ is found in the similar manner: $Pr(Y=n)$ = *Pr(n-1 samples are classified as negative and one sample is classified as positive)+Pr(n samples are classified as negative)*. It follows that:

$$Pr(Y=n) = (1-C)^{n-1}\ .$$

The probability function of Y obtained above is easily verified to be valid since:

$$\sum_{k=1}^{n} Pr(Y=k) = \sum_{k=1}^{n-1} C(1-C)^{k-1}+(1-C)^{n-1} = C\frac{1-(1-C)^{n-1}}{1-(1-C)}+(1-C)^{n-1} = 1\ .$$

The formula for the geometric series can be used in the above expression. This concludes the proof of Result 8.■

RESULT 9:

$$E(Z_i) = \frac{A(1-p)}{F(1-C)} \qquad E(Z_i^2) = \frac{H}{1-C}$$

$$E((\sum_{i=1}^{k-1} Z_i)^2) = \frac{H(k-1)}{1-C}+\frac{(k-1)(k-2)[A(1-p)]^2}{[F(1-C)]^2}\ .$$

Proof: Random variable Z_i was defined in section 3.2 to be the number tests needed to classify a sample as negative given that it is eventually classified as negative.

$$Pr(Z_i=z) = \frac{Pr(a\ sample\ is\ classified\ as\ negative\ in\ z\ tests)}{Pr(a\ sample\ is\ classified\ as\ negative)} =$$

$$= \frac{Pr(a\ sample\ is\ classified\ as\ negative\ in\ z\ tests)}{1-C} .$$

The last expression was obtained by applying Result 5 and simplifying. The probability function of Z_i can now be written:

$$Pr(Z_i=z) = \frac{1}{1-C}[\prod_{j=1}^{z-1} pj_+(1)](1-pz_+(1)) \quad when\ z=2,3,\ldots,r1$$

$$and\ Pr(Z_i=z) = \frac{1 - p1_+(1)}{1-C} \quad when\ z=1 .$$

Expectation of Z_i is found using Result 3 and the simplifying expressions from section 3.2. Result 5 along with the definition of H leads to the desired form of the expected value of Z_i^2. The last part of the Result 9 can be verified as following:

$$E((\sum_{i=1}^{k-1} Z_i)^2) = E(\sum_{i=1}^{k-1} Z_i^2) + E(2\sum_{i=1}^{k-1}\sum_{\substack{j=1 \\ j\neq i}}^{k-1} Z_iZ_j) = (k-1)E(Z_i^2) + (k-1)(k-2)E^2(Z_i) .$$

Substitution of $E(Z_i)$ and $E(Z_i^2)$ in the above expression yields the final result.■

RESULT 10:

$$E(V_i) = r1C + \frac{(1-p)A}{F} \qquad E(V_i^2) = (r1)^2C + H .$$

Proof: Since V_i is the random variable which denotes the number of tests needed to classify a sample (i-th sample) its expected value can be written follows:

$$E(V_i) = rl\prod_{i=1}^{rl} pi_+(1) + \sum_{i=2}^{rl} i[\prod_{j=1}^{i-1} pj_+(1)](1-pi_+(1)) + 1 - pl_+(1) \ .$$

Application of Results 2 and 3 to the above formula as well as use of the simplifying expressions from section 3.2 leads to the desired result. Expectation of V_i^2 can be obtained in the similar fashion except for the fact that Result 4 should be used instead of Result 3.■

For each of the procedures discussed in the following sections (MS, MD and MI) the expected value and variance of the total number of tests needed to classify n items if a group of size n is tested are derived. For modified group testing procedures the following formula connects $E(T_n)$ and $E(T_n^+)$:

(11)

$$E(T_n) = [r + E(T_n^+)]\prod_{i=1}^{r} pi_+(n) + \sum_{i=2}^{r} i[\prod_{j=1}^{i-1} pj_+(n)](1-pi_+(n)) + 1 - pl_+(n).$$

Results 2 and 3 lead to the simplified form:

$$E(T_n) = [r + E(T_n^+)][1-(1-p)^n(1-B_n)] + \frac{(1-p)^n A_n}{F} \ . \qquad (12)$$

Formula similar to (11) connects $E(T_n^2)$, $E(T_n^+)$ and $E((T_n^+)^2)$ and after simplification using results 2 and 4 and abbreviations from section 3.2 it looks as follows:

$$E(T_n^2)=[r^2+2rE(T_n^+)+E((T_n^+)^2)][1-(1-p)^n(1-B_n)]+H_n \ . \qquad (13)$$

Both of the two above formulas are used in the derivation of variance. The form of $E(T_n^+)$ and $E((T_n^+)^2)$ depends on what group testing procedure we use.

Last three sections of this chapter present formulas for the performance measures for MI, MD and MS: expected number and variance of the total number of tests per sample. In the numerical analysis we also use the coefficient of variation (CV) as a quantity which reflects variability in units of average number of tests needed:

$$CV=\frac{\sqrt{variance}}{mean} \ . \qquad (14)$$

3.4 MODIFIED INDIVIDUAL TESTING (MI)

For MI we require each sample to be retested so that a sample is classified as positive only if it produces a positive result *r1* times. If a negative result is obtained at any stage of retesting a sample is treated as a negative. If *n* samples are tested according to MI the following is clearly true:

$$E\left(\frac{T_n}{n}\right)=E(T_1)=r1\prod_{i=1}^{r1} pi_+(1)+\sum_{i=2}^{r1} i(1-pi_+(1))\prod_{j=1}^{i-1} pj_+(1)+1-p1_+(1). \quad (15)$$

Results 2 and 3 lead to simplification:

$$E\left(\frac{T_n}{n}\right)=E(T_1)=r1\ C+\frac{A(1-p)}{F}\ . \quad (16)$$

The expression for the variance can be derived in the similar manner and simplified using Results 2 and 4 to the following:

$$var\left(\frac{T_n}{n}\right)=(r1)^2C\ +\ H\ . \quad (17)$$

Formulas (15) and (17) are used to calculate numerical values of the corresponding quantities and the results of these calculations are discussed in Chapter 5. Note that since MI is not a group testing procedure only three parameters are present in the formulas: p, pf_+ and $r1$. One more remark can be made with regard to the results in this section: if the probability of a false positive outcome is set to zero formula (16) simplifies to one, as expected.

3.5 MODIFIED DORFMAN'S PROCEDURE (MD)

In Dorfman's procedure a positive group test outcome results in individual testing of each sample. According to

MD positive classification of a group or a sample takes place when r ($n \geq 2$) or $r1$ ($n=1$) tests produce a positive result. After using Results 2 and 3 it follows that:

$$E(T_n^+) = n\,E(T_1) = n\left(r1\,C + \frac{A(1-p)}{F}\right) . \qquad (18)$$

The expression in (18) is actually an approximation since original prevalence rate is used in the derivation of $E(T_1)$ rather than the probability of an infected sample given that this sample came from a group classified as positive. It is explained at the beginning of the proof of Result 8 why we are forced to make this approximation for MS and since MS and MD are going to be compared we have to consistently make this approximation.

Combining formulas (12) and (18) leads to the expression for $E(T_n)$:

$$E(T_n) = [r + n\left(r1\,C + \frac{A(1-p)}{F}\right)][1-(1-p)^n(1-B_n)] + \frac{A(1-p)^n}{F} . \qquad (19)$$

Next $E(T_n^2)$ is obtained which is then used to find the variance. In order to use formula (13) to find this quantity we have to first derive the expression for $E((T_n^+)^2)$. The following derivation uses Results 2 and 4 in the last step:

$$E((T_n^+)^2) = E(n\,T_1^2) = n^2\,E((T_1)^2) = n^2[(r1)^2C + H] . \qquad (20)$$

Substitution of formulas (18) and (20) in (13) yields the final result:

$$E(T_n^2)=\{r^2+2rn(r1\ C+\frac{A(1-p)}{F})+ n^2[(r1)^2C+H]\}[1-(1-p)^n(1-B_n)]+H_n\ . \qquad (21)$$

Results (19) and (21) are used to find the expression for variance since: $Var(T_n)=E(T_n^2)-E^2(T_n)$. CV can be obtained using formula (14).

Formulas derived in this section are used in Chapter 5 to calculate the reciprocal of the expected number of tests per sample, which is referred to as the expected number of samples classified by a single test, and corresponding coefficient of variation.

Expectation for MD, derived in formula (19), divided by the group size simplifies to the corresponding expression obtained by Dorfman (formula (2) in Chapter 2) if pf_+ is set to zero since then $A=F=r=r1=1$, $C=p$ and $B=0$.

3.6 MODIFIED STERRETT'S PROCEDURE (MS)

In order to apply formula (12) to find the expected number of tests per sample for MS we have to derive the expression for $E(T_n^+)$ first. To do this we make use of the random variables defined in section 3.2. Careful examination of the original Sterrett's process (Figure 1) with modification of

retesting for positive classification and definitions of these random variables lead to the following:

$$T_n^+ = \begin{cases} X_1+T_{n-1} & ,if\ Y = 1\ , \\ \sum_{i=1}^{Y-1} Z_i+X_Y+T_{n-Y} & ,if\ Y = 2,3,\ldots,n-1\ , \\ \sum_{i=1}^{n-1} Z_i+V_n & ,if\ Y = n\ . \end{cases} \tag{22}$$

Consequently, we can write the expression for $E(T_n^+)$:

$$E(T_n^+) = E(X_1+T_{n-1})Pr(Y=1)+\sum_{k=2}^{n-1} E(\sum_{i=1}^{k-1} Z_i+X_k+T_{n-k})Pr(Y=k)+ \\ + E(\sum_{i=1}^{n-1} Z_i+V_n)Pr(Y=n)\ . \tag{23}$$

Substitution of Results 7,8,9 and 10 in the above formula yields the following expression:

$$E(T_n^+) = [rl + E(T_{n-1})]C+\sum_{k=2}^{n-1}[(k-1)\frac{A(1-p)}{F(1-C)} + rl + \\ E(T_{n-k})]C(1-C)^{k-1}+[(n-1)\frac{A(1-p)}{F(1-C)} + rlC+\frac{(1-p)A}{F}](1-C)^{n-1}. \tag{24}$$

This expression can be simplified by first separating constant terms from the terms which involve expectations and then simplifying the constant part. After several steps of algebraic manipulation the following expression is obtained:

$$E(T_n^+) = P+\sum_{k=1}^{n-1} E(T_{n-k})C(1-C)^{k-1}\ . \tag{25}$$

The formula for $E(T_n)$ can then be found using formula (12) and simplifying:

$$E(T_n) = (r+P)[1-(1-p)^n(1-B_n)] + \frac{A_n(1-p)^n}{F} + \\ + C[1-(1-p)^n(1-B_n)]\sum_{k=1}^{n-1} E(T_{n-k})(1-C)^{k-1} \quad . \tag{26}$$

The derivation of the variance of the total number of tests requires the expression for $E((T_n)^2)$. Following procedure which led to formulas (11) and (12), applying Results 2 and 4 and making use of the simplifying expressions from section 3.2 lead to the formula:

$$E(T_n^2) = [r^2+2rE(T_n^+)+E((T_n^+)^2)][1-(1-p)^n(1-B_n)] + H_n \quad . \tag{27}$$

Using the independence of random variables X_i, Z_i, V_i and T_i it follows that:

$$E((T_n^+)^2) = [E(X_1)+2E(X_1)E(T_{n-1}) + E(T_{n-1}^2)]Pr(Y=1) + \\ \sum_{k=2}^{n-1}\{E((\sum_{i=1}^{k-1} Z_i)^2)+2(k-1)E(Z_i)[E(X_k)+ \\ E(T_{n-k})] + E(X_k^2)+2E(X_k)E(T_{n-k})+E(T_{n-k}^2)\}Pr(Y=k) + \\ +[E((\sum_{i=1}^{n-1} Z_i)^2)+2(n-1)E(Z_i)E(V_n)+E(V_n^2)]Pr(Y=n) \quad . \tag{28}$$

Formula (28) can be simplified by applying Results 7,8,9 and 10 to it and separating constant terms from terms which involve expectations. Extensive algebraic manipulation will lead to the following expression:

$$E((T_n^+)^2) = S + \sum_{k=1}^{n-1}\{E(T_{n-k}^2) + [\frac{2(k-1)A(1-p)}{F(1-C)} + 2rl]E(T_{n-k})\}C(1-C)^{k-1}. \tag{29}$$

Substituting formulas (25) and (29) in (27) and simplifying bring us to the final result:

$$E(T_n^2) = H_n+[1-(1-p)^n(1-B_n)](r^2+2rP+S)+ \qquad (30)$$
$$+\sum_{k=1}^{n-1}[1-(1-p)^n(1-B_n)]C(1-C)^{k-1}E(T_{n-k}^2)+$$

$$+\sum_{k=1}^{n-1}2[1-(1-p)^n(1-B_n)][\frac{A(k-1)(1-p)}{F(1-C)}+r+rl]C(1-C)^{k-1}E(T_{n-k}) \ .$$

The variance is obtained by subtracting the square of expression (26) from expression (30).

It can be illustrated that the approach which is taken here to derive the formula for the expected number of samples per test can be applied to the situation when $pf_+=0$ and the resulting numerical values will be the same as those obtained by Sterrett [13]. In order to verify this, the formula presented by Sterrett is evaluated first. Numbers reported in his thesis can not be used since Sterrett employed an approximation to evaluate his procedure. The exact formula for the expected number of samples per test is the following:

$$\frac{E(n,p)}{n} = \frac{1}{n}\sum_{i=0}^{n}[\binom{n}{i}p^i(1-p)^{n-i}(\frac{i}{i+1}n+i+1+\frac{i}{i+1}-2i\frac{1}{n})] \ . \qquad (31)$$

In order to obtain our equivalent of this formula some modifications have to be made. We can not simply let $pf_+=0$ and simplify in this case for the following reason: when tests are considered to be flawless and individual testing is performed

on the defective group of size n the last sample is not tested if $n-1$ samples turned out to be negative, on the other hand, if there is a chance of a false positive classification and the same situation occurs last sample must be tested. Therefore, in order to make our procedure compatible with Sterrett's derivations we have to account for this difference. First of all the distribution of Y will change and, secondly, $E(V_n)$ will not appear in the expression for $E(T_n)$. The distribution of Y can now be derived as follows.

For $k=1,2,\ldots,n-1$ $Pr(Y=k)=Pr(k-1$ *samples are classified as negative and one is classified as positive| Group is classified as positive)* $=Pr(k-1$ *samples are classified as negative and one is classified as positive)(Pr(Group is classified as positive))*$^{-1}$ since when classification errors are not present every group containing a positive sample produces a positive test outcome. Consequently,

$$Pr(Y = k) = \frac{p(1-p)^{k-1}}{1-(1-p)^n},\ k=1,2,\ldots,n-1. \qquad (32)$$

Similarly, $Pr(Y=n)=Pr(n-1$ *samples are classified as negative| Group is classified as positive)*$=pPr(n-1$ *samples are classified as negative)(Pr(Group is classified as positive))*$^{-1}$ since group of size n which contains $n-1$ negative samples is positive only if its n-th member is positive when $pf_+=pf_-=0$. This implies the following:

$$Pr(Y=n) = \frac{p(1-p)^{n-1}}{1-(1-p)^n} . \qquad (33)$$

By combining formulas (32) and(33) we get the following new distribution of Y:

$$Pr(Y=k) = \frac{p(1-p)^{k-1}}{1-(1-p)^n} \quad ,k=1,2,\ldots,n. \qquad (34)$$

New expression for $E(T_n)$ can now be derived just like at the beginning of this section but with pf_+ and V_n set to zero and with the distribution of Y presented in (34). It was verified numerically that the resulting expression divided by n produces the same results as formula (31). For example,

p=0.25 produces n^*=3 and $E(T_{n^*}/n^*)$=0.839;

p=0.10 produces n^*=5 and $E(T_{n^*}/n^*)$=0.523;

p=0.01 produces n^*=15 and $E(T_{n^*}/n^*)$=0.152.

It was mentioned earlier that the expression we derived for the expected number of tests per sample (formula (26)) is actually an approximation due to the fact that distribution of Y used in this expression is not exact. An obvious question arises: how good is our approximation? Since for the situation when pf_+=0 we know the exact results it seems reasonable to approximate them in a way similar to the one used for MS and observe the difference in numerical outcomes. Ignoring the information about the group being positive and setting pf_+ to

zero results in the following distribution of Y:

$$Pr(Y=k) = \begin{cases} p(1-p)^{k-1} & ,if\ k=1,2,\ldots,n-1. \\ (1-p)^{n-1} & ,if\ k=n. \end{cases} \tag{35}$$

This distribution of Y was used to calculate approximate expected values and the following result were obtained:

p=0.25: Exact$(E(T_{n^*}/n^*))$=0.839 and Approx.$(E(T_{n^*}/n^*))$=0.773;

p=0.10: Exact$(E(T_{n^*}/n^*))$=0.523 and Approx.$(E(T_{n^*}/n^*))$=0.513;

p=0.01: Exact$(E(T_{n^*}/n^*))$=0.152 and Approx.$(E(T_{n^*}/n^*))$=0.183.

The fact that the values presented above are quite close suggests that our approximation is probably a good one.

Results derived in this section are used to obtain numerical values which are presented and discussed in Chapter 5. In the next chapter the procedure for screening blood for HIV currently used by Canadian Red Cross is discussed.

Chapter 4

HIV BLOOD SCREENING PROCEDURE USED BY CANADIAN RED CROSS

As mentioned in Chapter 1 the Canadian Red Cross attempts to reduce the probability of collecting an infected sample of blood by requesting each donor to fill out a questionnaire which is designed to identify people who come from a 'high risk' pool (Figure 3). Each of the potential donors is then interviewed by a staff member of the Red Cross. Once the blood is collected, it is transferred to one of the Red Cross testing laboratories, where extensive screening of blood for various infections is carried out. HIV screening is a critical component of this procedure. The testing procedure used for HIV screening is described next.

During screening for HIV, blood samples are tested individually but simultaneously, eighty nine at one time. Ninety six samples, four of which are positive controls and three are negative controls, are put in separate wells on a single platform and simultaneous testing is performed using ELISA. A nontechnical description of ELISA is given by Wein and Zenios [9, pages 4-5]. The cut-off value for positive and negative classification is determined using the mean of the normalized OD readings of negative controls plus 0.25. OD stands for the optical density level (ELISA outcome) which is normalized as follows:

$$NORMALIZED(OD) = \frac{OD - Min}{Max - Min},$$

where *Min* and *Max* are the smallest and the largest OD levels in the data. A sample that produces a positive result on the first test is classified as "initially reactive" and it is then retested in duplicate. If at least one of the retests is positive the blood sample is classified as "repeatedly reactive" and is sent for confirmatory retesting to a laboratory in Ottawa where an expensive and more accurate Western Blot test is used to determine whether the blood is indeed infected. Regardless of the outcome of the Western Blot, repeatedly reactive blood samples are never used. Initially reactive samples which produce negative outcomes for both retests are considered uninfected and are passed on to

the hospitals for use. Figure 2 gives a graphical description of the procedure just discussed. In what follows this procedure is denoted by CP.

CP is intuitively reasonable but from a statistical view point it defies logic because it gives the first negative outcome much more credibility than the second and the third. We have not been able to find any statistical basis for CP in published literature. Our evaluation of the performance of CP is presented next. It is compared with performances of MS and MD in Section 5.1.

We will derive expressions for $E(T_N)$ and $var(T_N)$. In order to find these expressions we define the following quantities:

$$I = \begin{cases} 0, & \textit{if a sample produces negative result on first ELISA test.} \\ 1, & \textit{otherwise.} \end{cases}$$

$Pr(I=1)$ = *Pr(first ELISA test on a sample is positive) and*

$Pr(I=0) = 1-Pr(I=1)$.

Consequently, $E(T_N/N)$ and $var(T_N/N)$ are obtained:

$$E\left(\frac{T_N}{N}\right) = \frac{N+2NE(I)}{N} = 1+2Pr(I=1) = 1+2[p+(pf_+)(1-p)]$$

$$var\left(\frac{T_N}{N}\right) = \frac{var(N+2(\textit{number of samples out of N which are initially positive}))}{N^2} =$$

$$= \frac{4var(I)}{N} = \frac{4Pr(I=1)(1-Pr(I=1))}{N} =$$

$$= \frac{4[p+(pf_+)(1-p)][1-p-(pf_+)(1-p)]}{N}.$$

Now performance of CP can be evaluated for any given pair of parameters (p, pf_+) and different values of N for variance. The following values were reported by Ontario Hospitals Association: p=0.00009, *Pr(repeatedly reactive sample)*=0.002 [11]. Using the latter value we can find the corresponding probability of a false positive outcome:

Pr(repeatedly reactive sample) = 2Pr(initially reactive sample)Pr(second ELISA test is positive|first ELISA test was positive)Pr(second ELISA test is negative|first ELISA test was positive)+ Pr(initially reactive sample)(Pr(second ELISA test is positive| first ELISA test was positive))^2.

In the expression above we used the fact that retesting is done simultaneously rather than sequentially. Using the formula for probabilities derived earlier in Result 1 and simplifying the resulted expression produces an equation which can be solved using Newton's method. If *Pr(repeatedly reactive sample)*=0.002 [11] it can be found using MAPLE that pf_+ = 0.031. Values of performance measures for CP for several other parameter pairs are given and discussed in section 5.1.

Chapter 5, which follows next, contains numerical results and final conclusions.

Chapter 5

DISCUSSION OF RESULTS

5.1 NUMERICAL RESULTS

In this section MI, MD and MS are compared in terms of expected number of samples classified by a single test ($1/E(T_n/n)$) and the coefficient of variation (CV). These quantities are calculated for various parameter pairs (p, pf_+). Table 2 illustrates some results. The first pair of values comes from reports of the Ontario Hospitals Association [11]. The second pair results when threshold is moved to an extreme value and as a consequence pf_+ is very high. The remaining pairs illustrate some instances for which circumstances are not favourable for group testing due to a high prevalence rate. It is clear from the results that both MD and MS are superior to MI and that there is not much difference in performance of MD and MS. Coefficients of variation are much lower for MI which is to be expected since

greater variability is naturally present when group testing is used.

Table 2 PERFORMANCE MEASURES AND OPTIMAL GROUP SIZES FOR MI, MD AND MS

p pf_+	MI		MD			MS		
	$1/E(T_n/n)$	CV	$1/E(T_n/n)$	CV	n*	$1/E(T_n/n)$	CV	n*
0.00009 0.032	0.97	0.18	50.65	5.23	106	50.77	4.92	106
0.00009 0.98	0.02	0.10	1.01	7.24	105	1.01	5.32	105
0.01 0.0005	0.99	0.10	5.07	1.52	10	5.18	1.56	11
0.1 0.006	0.90	0.28	1.57	0.88	4	1.62	0.83	4
0.1 0.01	0.89	0.30	1.57	0.88	4	1.60	0.83	4

The similarities of the shapes and values of the expected number of samples classified by a test become apparent from Figures 5 and 6. For fixed parameter pairs, the plots corresponding to MD and MS look almost identical. All graphs of $1/E(T_n/n)$ versus n follow the pattern of first increasing, reaching the maximum and then decreasing except for a few of instances of small increases. The n^* reported in Table 2 is a value of n from the range (0,300) which yields the maximum value of $1/E(T_n/n)$. Although we can not strictly rule out the possibility that even larger $1/E(T_n/n)$ values exist for some $n>300$, we have strong reasons for not considering such large group sizes. In most geographical regions of Canada, the

number of blood samples collected per day is less than 300. Since blood has limited shelf life, there is a great urgency to test it immediately and, if found safe, to make it available for use by hospitals. Thus, waiting a day or more to form an appropriate group size is not an acceptable option. Equally important is the fact that group testing is not likely to be feasible for very large group sizes owing to dilution effect.

Figures 7 and 8 illustrate the behaviour of the maximum value of $1/E(T_n/n)$ as p and pf_+ vary. Once again the patterns are similar for MD and MS. The nature of the relationship seems to be linear when pf_+ is changing and hyperbolic when p is changing but in both cases increase in p or pf_+ results in decrease in the expected number of samples classified by a single test. This negative relationship is expected since higher prevalence rate and the probability of a false positive outcome inevitably results in larger number of positive groups and hence a greater number of retests. The plots also indicate that most of the time small changes in the values of p and pf_+ do not effect the value of $1/E(T_{n^*}/n^*)$ by much. Only exception is the case when p is very small. Absence of drastic changes in the value of $1/E(T_{n^*}/n^*)$ in response to small shifts in p and pf_+ is important since in practice the exact values of parameters are seldom known. The behaviour of the optimal group size, n^*, as p and pf_+ vary is explored in Figure 11. The

patterns observed are not monotonic and we have not been able to explain the reason behind these patterns.

It was suggested by Litvak, Tu and Pagano [10] that a sample size of fifteen is often convenient to use in group testing. Suppose it is not possible to use optimal group sizes shown in Table 2 because they are too large and that a group size of fifteen is used instead. Table 3 shows that even in this case group testing is more efficient and MD and MS produce similar results. It would have been interesting to see how our results compare with those obtained by Litvak, Tu and Pagano. Unfortunately, their paper does not contain any tables. Only plots are used for illustrations and as a result any comparisons are subject to reading inaccuracies. Furthermore, Litvak, Tu and Pagano use pairs: $pf_+=pf_-=0.005$, while in our model $pf_-=0$. Due to these factors our comparisons may not be precise but it seems that MS, MD and procedures considered by Litvak, Tu and Pagano have similar performances.

Table 3 EXPECTED NUMBER OF SAMPLES CLASSIFIED BY A SINGLE TEST IF GROUP SIZE OF FIFTEEN IS USED

p pf_+	MI	MD	MS
0.00009 0.031	0.97	14.18	14.20
0.01 0.0005	0.99	4.80	4.97
0.00009 0.98	0.02	0.29	0.29

An important feature in the procedures discussed here is the number of retests that are performed on a group or on a sample. Figures 9 and 10 illustrate the effect of changing p, pf_+ and n on the value of r. As the first two parameters gradually increase, the number of retests changes in a stepwise manner. It stays unaffected for awhile and then jumps one unit up (if pf_+ is increasing) or down (if p is increasing). This behaviour of r is expected since higher probability of a false positive outcome requires more extensive retesting, while higher prevalence rate suggests that the positively tested group is likely to be a true positive and, therefore, the number of retests should decrease. The effect of group size on the number of retests is illustrated for the cases when p is very small (p=0.00009) and pf_+ is 0.032 and 0.8. In both these situations r decreases as n increases. The value of r seems to stay constant when pf_+=0.032.

In an application like ours, it may be desirable to avoid higher variability even at some cost. Our analysis show that coefficient of variation increases as n increases (Figures 12a,12b,12c and 12d). If accuracy is very important, group sizes used can be smaller than the optimal values which will result in lower CV and higher $E(T_n/n)$.

Table 4 PERFORMANCE MEASURES FOR CP, MD, AND MS

p	CP		MD		MS	
pf_+	$1/E(T_n/n)$	CV	$1/E(T_n/n)$	CV	$1/E(T_n/n)$	CV
0.00009 0.031	0.94	0.04	50.77	4.92	50.65	5.23
0.00009 0.3	1.06	0.06	36.15	4.97	36.06	5.93
0.00009 0.8	2.60	0.03	10.17	5.05	10.15	6.95

It was discussed in Chapter 4 how CP can be evaluated for given parameter values. Table 4 illustrates the numerical results. First set of parameters reflects the current situation while two other parameter pairs correspond to situations in which we want to be sure that the probability of a false negative outcome is nearly zero. Therefore , we have set pf_+ quite large. Numerical results show that the expected number of samples classified by a single test is much higher for MS and MD in comparison with CP. The coefficient of variation , however, is smaller for CP.

5.2 RECOMMENDATIONS

Based on the results obtained in this thesis, it is our conclusion that group testing is a more efficient than the procedure currently used by the Canadian Red Cross. Since MD

and MS produce similar results, it is recommended that Modified Dorfman's procedure be used. It is easier to implement and, therefore, reduces the possibility of human error. If blood screening can be performed using robots or other mechanized devices and even small savings are important MS, may be considered instead of MD.

5.3 FUTURE DIRECTIONS

In these thesis we have discussed and compared several procedures suitable for screening blood for HIV. From managerial and statistical point of view the goal of future research is to develop an even more efficient group testing procedure. It may be possible to combine the strategies, discussed in these thesis, to produce a more efficient protocol. For example, every time we have a positively classified group we may want to consider several ways of action: 1)test all samples individually (Dorfman's approach [5]); 2)perform individual testing only until one of the group members is classified as positive (Sterrett's approach [13]); 3)draw a subgroup from this group and test it (Sobel and Groll's approach [12]). Modelling of such procedure will probably make use of the dynamic programming approach and will have to address the problem of classification errors.

Biological research is necessary to establish the feasibility and possible effects of pooling blood samples when

newly developed tests are considered for HIV screening (for example, PCR).

And finally, it is imperative that Canadian Red Cross uses the most efficient and safest methods when screening blood for various diseases, in particular for HIV. Consequently, difficulties surrounding implementation of group testing strategies need to be researched and alleviated. Senior managers as well as technicians need to participate in suitably modifying these procedures to reduce the risk of human/handling errors. Only then can the full potential of this research be realized.

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APPENDIX A

Tables

Table 5 Performance measures for MD for various group sizes when prevalence rate is 0.00009 and probability of a false positive outcome is 0.031. (* denotes the optimal group size.)

n	1/E(Tn/n)	VAR(Tn/n)	CV(Tn/n)
2	1.936	0.009	0.188
3	2.902	0.005	0.201
4	3.866	0.003	0.219
5	4.828	0.003	0.243
6	5.786	0.002	0.271
7	6.741	0.002	0.304
8	7.692	0.002	0.34
9	8.638	0.002	0.38
10	9.579	0.002	0.423
11	10.515	0.002	0.468
12	11.444	0.002	0.516
13	12.367	0.002	0.566
14	13.283	0.002	0.617
15	14.192	0.002	0.671
16	15.094	0.002	0.725
17	16.003	0.002	0.753
18	16.889	0.002	0.81
19	17.767	0.002	0.868
20	18.635	0.002	0.927
21	19.494	0.003	0.987
22	20.343	0.003	1.049
23	21.182	0.003	1.11
24	22.01	0.003	1.173
25	22.828	0.003	1.236
26	23.634	0.003	1.3
27	24.43	0.003	1.365
28	25.213	0.003	1.43
29	25.985	0.003	1.495
30	26.745	0.003	1.561
31	27.493	0.004	1.627
32	28.229	0.004	1.693
33	28.952	0.004	1.759
34	29.662	0.004	1.826
35	30.359	0.004	1.892
36	31.044	0.004	1.958
37	31.716	0.004	2.025
38	32.374	0.004	2.091
39	33.02	0.004	2.157
40	33.652	0.004	2.223
41	34.271	0.004	2.289
42	34.876	0.005	2.355
43	35.469	0.005	2.42
44	36.048	0.005	2.485
45	36.614	0.005	2.549
46	37.166	0.005	2.614
47	37.705	0.005	2.677
48	38.231	0.005	2.741
49	38.744	0.005	2.804
50	39.244	0.005	2.866
51	39.731	0.005	2.928
52	40.205	0.006	2.989
53	40.666	0.006	3.049
54	41.114	0.006	3.11
55	41.55	0.006	3.169
56	41.973	0.006	3.228
57	42.384	0.006	3.286
58	42.782	0.006	3.344
59	43.168	0.006	3.4
60	43.543	0.006	3.457
61	43.905	0.006	3.512
62	44.256	0.007	3.567
63	44.595	0.007	3.621
64	44.922	0.007	3.674
65	45.239	0.007	3.727
66	45.544	0.007	3.779
67	45.839	0.007	3.83
68	46.122	0.007	3.88
69	46.395	0.007	3.929
70	46.658	0.007	3.978
71	46.911	0.007	4.026
72	47.153	0.007	4.074
73	47.385	0.008	4.12
74	47.608	0.008	4.166
75	47.821	0.008	4.211
76	48.025	0.008	4.255
77	48.22	0.008	4.299
78	48.406	0.008	4.341
79	48.583	0.008	4.383
80	48.751	0.008	4.425
81	48.911	0.008	4.465
82	49.062	0.008	4.505
83	49.206	0.009	4.544
84	49.341	0.009	4.582
85	49.469	0.009	4.62
86	49.589	0.009	4.656
87	49.702	0.009	4.692
88	49.807	0.009	4.728
89	49.906	0.009	4.762
90	49.997	0.009	4.796
91	50.082	0.009	4.829
92	50.16	0.009	4.862
93	50.232	0.009	4.894
94	50.298	0.01	4.925
95	50.357	0.01	4.956
96	50.411	0.01	4.985
97	50.458	0.01	5.015
98	50.5	0.01	5.043
99	50.537	0.01	5.071
100	50.568	0.01	5.098
101	50.594	0.01	5.125
102	50.614	0.01	5.151
103	50.63	0.01	5.177

cont'd Table 5

n	1/E(Tn/n)	VAR(Tn/n)	CV(Tn/n)
104	50.641	0.011	5.201
105	50.648	0.011	5.226
106*	50.649	0.011	5.249
107	50.647	0.011	5.273
108	50.64	0.011	5.295
109	50.629	0.011	5.317
110	50.614	0.011	5.339
111	50.594	0.011	5.36
112	50.572	0.011	5.38
113	50.545	0.011	5.4
114	50.515	0.012	5.419
115	50.481	0.012	5.438
116	50.444	0.012	5.457
117	50.403	0.012	5.475
118	50.36	0.012	5.492
119	50.313	0.012	5.509
120	50.263	0.012	5.525
121	50.211	0.012	5.541
122	50.156	0.012	5.557
123	50.097	0.012	5.572
124	50.037	0.012	5.587
125	49.974	0.013	5.601
126	49.908	0.013	5.615
127	49.84	0.013	5.629
128	49.77	0.013	5.642
129	49.697	0.013	5.654
130	49.622	0.013	5.667
131	49.546	0.013	5.678
132	49.467	0.013	5.69
133	49.386	0.013	5.701
134	49.304	0.013	5.712
135	49.219	0.014	5.722
136	49.133	0.014	5.733
137	49.046	0.014	5.742
138	48.956	0.014	5.752
139	48.866	0.014	5.761
140	48.773	0.014	5.77
141	48.68	0.014	5.778
142	48.585	0.014	5.786
143	48.488	0.014	5.794
144	48.391	0.014	5.802
145	48.292	0.014	5.809
146	48.192	0.015	5.816
147	48.091	0.015	5.823
148	47.989	0.015	5.829
149	47.886	0.015	5.835
150	47.782	0.015	5.841

Table 6 Performance measures for MS for various group sizes when prevalence rate is 0.00009 and probability of a false positive outcome is 0.031. (* denotes the optimal group size.)

n	1/E(Tn/n)	VAR(Tn/n)	CV(Tn/n)	r
2	1.937	0.009	0.186	4
3	2.904	0.005	0.199	4
4	3.869	0.003	0.217	4
5	4.831	0.002	0.241	4
6	5.79	0.002	0.269	4
7	6.745	0.002	0.302	4
8	7.696	0.002	0.338	4
9	8.643	0.002	0.377	4
10	9.584	0.002	0.419	4
11	10.521	0.002	0.464	4
12	11.451	0.002	0.511	4
13	12.374	0.002	0.56	4
14	13.291	0.002	0.611	4
15	14.201	0.002	0.664	4
16	15.117	0.002	0.689	4
17	16.012	0.002	0.744	3
18	16.899	0.002	0.8	3
19	17.778	0.002	0.857	3
20	18.647	0.002	0.915	3
21	19.506	0.003	0.974	3
22	20.356	0.003	1.035	3
23	21.195	0.003	1.095	3
24	22.025	0.003	1.157	3
25	22.843	0.003	1.219	3
26	23.65	0.003	1.282	3
27	24.446	0.003	1.346	3
28	25.231	0.003	1.41	3
29	26.003	0.003	1.474	3
30	26.764	0.003	1.538	3
31	27.513	0.003	1.603	3
32	28.249	0.003	1.668	3
33	28.973	0.004	1.734	3
34	29.684	0.004	1.799	3
35	30.382	0.004	1.864	3
36	31.068	0.004	1.93	3
37	31.741	0.004	1.995	3
38	32.4	0.004	2.06	3
39	33.046	0.004	2.125	3
40	33.68	0.004	2.19	3
41	34.3	0.004	2.255	3
42	34.906	0.004	2.319	3
43	35.5	0.005	2.383	3
44	36.08	0.005	2.447	3
45	36.647	0.005	2.511	3
46	37.201	0.005	2.574	3
47	37.741	0.005	2.637	3
48	38.269	0.005	2.699	3
49	38.783	0.005	2.761	3
50	39.284	0.005	2.822	3
51	39.772	0.005	2.883	3
52	40.247	0.005	2.943	3

n	1/E(Tn/n)	VAR(Tn/n)	CV(Tn/n)	r
53	40.71	0.005	3.002	3
54	41.16	0.006	3.061	3
55	41.597	0.006	3.12	3
56	42.021	0.006	3.178	3
57	42.434	0.006	3.235	3
58	42.834	0.006	3.291	3
59	43.222	0.006	3.347	3
60	43.598	0.006	3.403	3
61	43.962	0.006	3.457	3
62	44.314	0.006	3.511	3
63	44.655	0.006	3.564	3
64	44.984	0.006	3.616	3
65	45.302	0.007	3.668	3
66	45.61	0.007	3.719	3
67	45.906	0.007	3.769	3
68	46.191	0.007	3.819	3
69	46.466	0.007	3.867	3
70	46.731	0.007	3.915	3
71	46.985	0.007	3.963	3
72	47.229	0.007	4.009	3
73	47.464	0.007	4.055	3
74	47.689	0.007	4.1	3
75	47.904	0.007	4.144	3
76	48.11	0.008	4.188	3
77	48.307	0.008	4.231	3
78	48.494	0.008	4.273	3
79	48.673	0.008	4.314	3
80	48.844	0.008	4.354	3
81	49.006	0.008	4.394	3
82	49.159	0.008	4.433	3
83	49.305	0.008	4.472	3
84	49.442	0.008	4.509	3
85	49.572	0.008	4.546	3
86	49.695	0.009	4.582	3
87	49.81	0.009	4.618	3
88	49.917	0.009	4.652	3
89	50.018	0.009	4.687	3
90	50.112	0.009	4.72	3
91	50.199	0.009	4.753	3
92	50.279	0.009	4.785	3
93	50.353	0.009	4.816	3
94	50.421	0.009	4.847	3
95	50.482	0.009	4.877	3
96	50.538	0.009	4.906	3
97	50.588	0.01	4.935	3
98	50.632	0.01	4.963	3
99	50.671	0.01	4.991	3
100	50.704	0.01	5.018	3
101	50.732	0.01	5.044	3
102	50.755	0.01	5.069	3
103	50.773	0.01	5.095	3

cont'd Table 6

n	1/E(Tn/n)	VAR(Tn/n)	CV(Tn/n)	r
104	50.787	0.01	5.119	3
105	50.795	0.01	5.143	3
106*	50.799	0.01	5.166	3
107	50.799	0.01	5.189	3
108	50.794	0.011	5.211	3
109	50.785	0.011	5.233	3
110	50.772	0.011	5.254	3
111	50.755	0.011	5.275	3
112	50.735	0.011	5.295	3
113	50.71	0.011	5.315	3
114	50.682	0.011	5.334	3
115	50.651	0.011	5.353	3
116	50.616	0.011	5.371	3
117	50.577	0.011	5.388	3
118	50.536	0.011	5.406	3
119	50.491	0.012	5.422	3
120	50.444	0.012	5.439	3
121	50.394	0.012	5.454	3
122	50.34	0.012	5.47	3
123	50.284	0.012	5.485	3
124	50.226	0.012	5.499	3
125	50.165	0.012	5.514	3
126	50.101	0.012	5.527	3
127	50.035	0.012	5.541	3
128	49.967	0.012	5.554	3
129	49.896	0.012	5.566	3
130	49.824	0.013	5.578	3
131	49.749	0.013	5.59	3
132	49.672	0.013	5.601	3
133	49.594	0.013	5.613	3
134	49.513	0.013	5.623	3
135	49.431	0.013	5.634	3
136	49.347	0.013	5.644	3
137	49.261	0.013	5.653	3
138	49.174	0.013	5.663	3
139	49.085	0.013	5.672	3
140	48.995	0.013	5.681	3
141	48.903	0.014	5.689	3
142	48.81	0.014	5.697	3
143	48.716	0.014	5.705	3
144	48.62	0.014	5.712	3
145	48.523	0.014	5.72	3
146	48.425	0.014	5.727	3
147	48.326	0.014	5.733	3
148	48.226	0.014	5.74	3
149	48.124	0.014	5.746	3
150	48.022	0.014	5.752	3

APPENDIX B

Figures

Figure 1 Individual and group testing procedures when applied to a group of samples of size *n*.

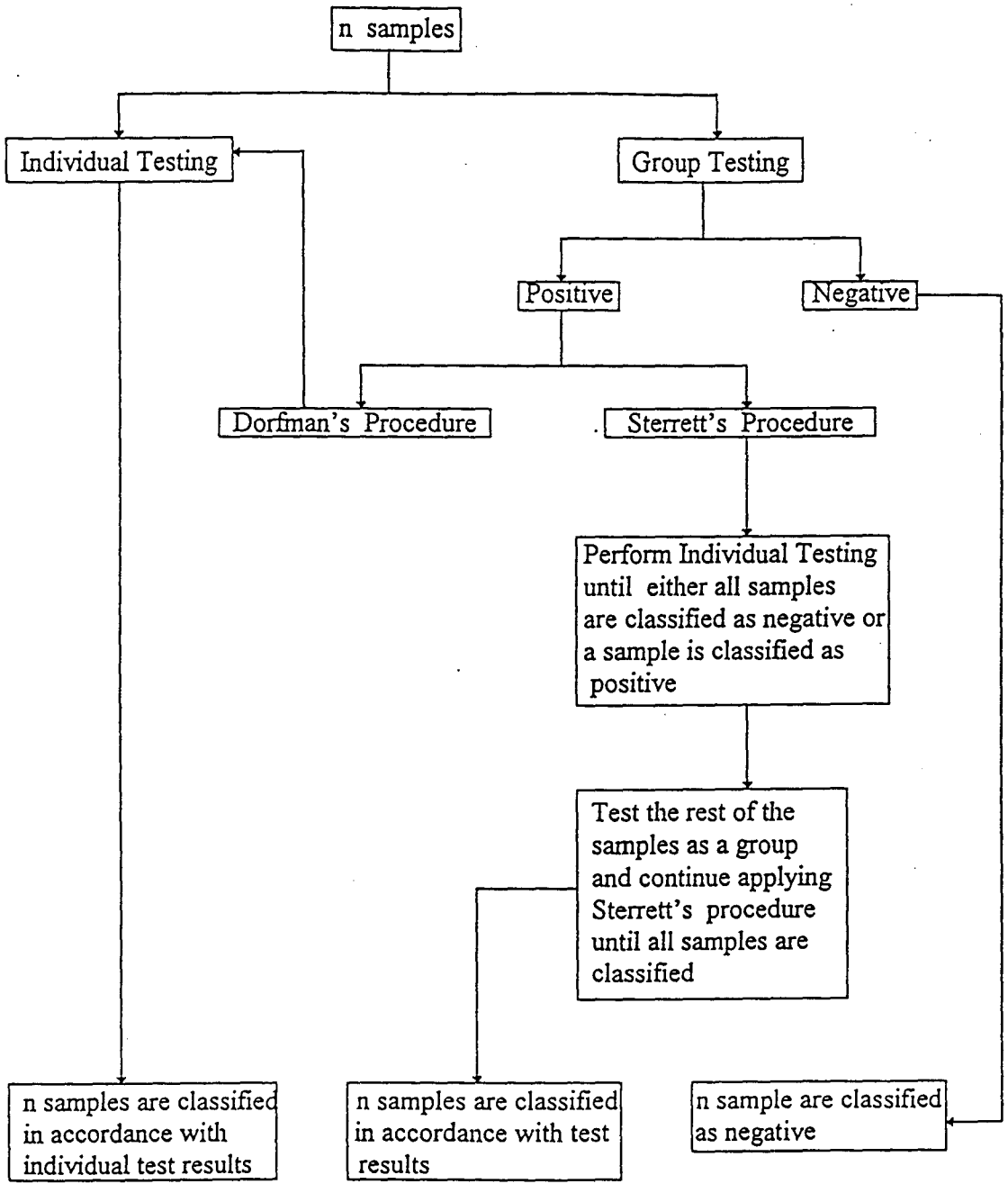


Figure 2 Procedure for screening blood for HIV currently used by the Canadian Red Cross.

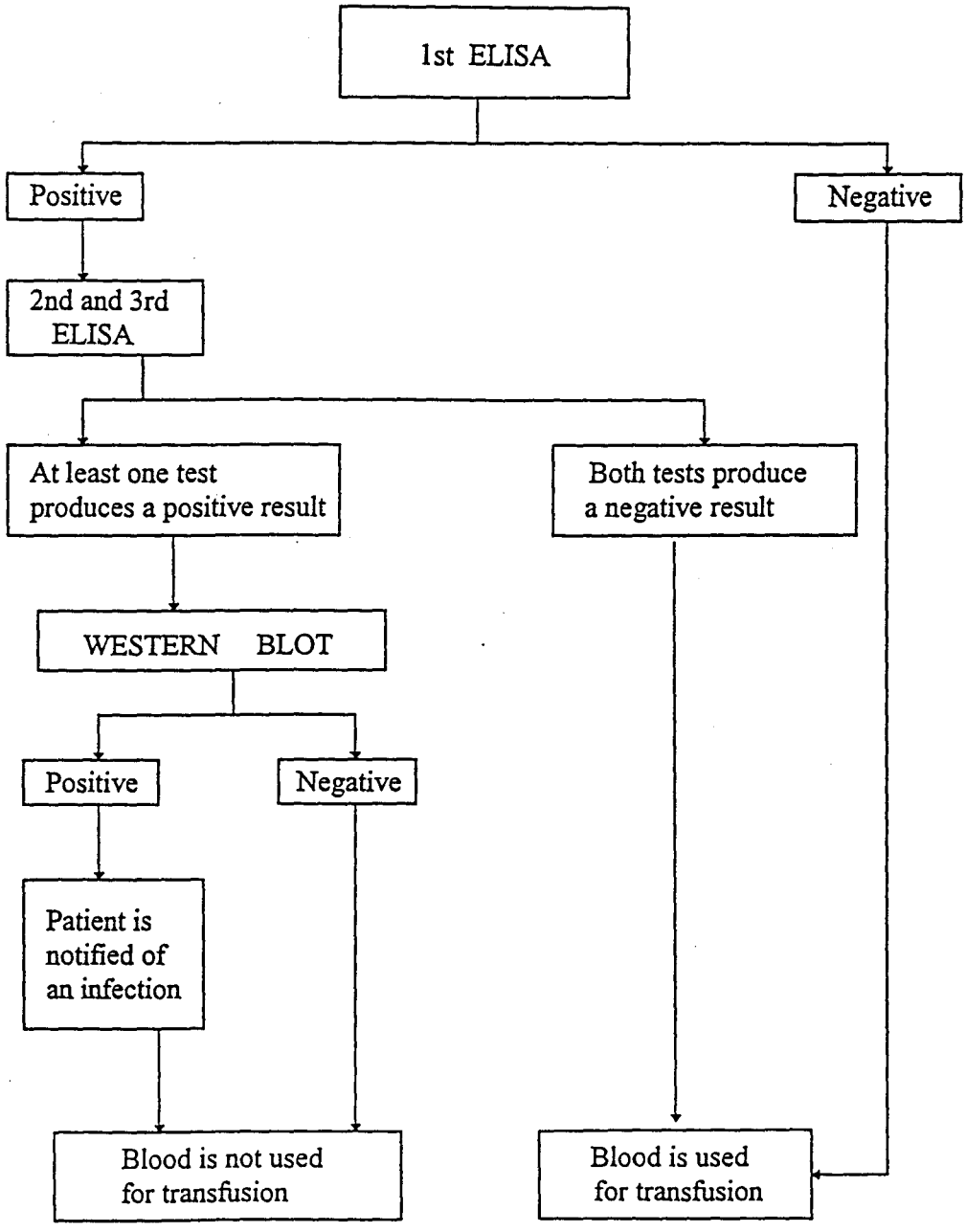


Figure 3 Questionnaire used by the Canadian Red Cross.

Thank you for attending today's blood donor clinic. Before you can donate, we must make sure that you are in good health. This is for your protection and for the protection of those who will receive your blood.

ANSWER ALL QUESTIONS YES OR NO

	Yes	No
1. a) In the last 3 days, have you taken any type of medicine or drugs (pills, needles), except birth control pills or vitamins?	☐	☐
b) Have you taken Accutane or Tegison for a skin disorder or growth hormone (human pituitary)?	☐	☐
2. Are you free from a cold, flu, infection or active allergy today?	☐	☐
3. Have you had a vaccination in the last 3 months or a rabies shot in the last year?	☐	☐
4. Have you had surgery in the last 6 months?	☐	☐
5. Have you had any of the following:		
a) epilepsy, coma, stroke, repeated seizures or fainting?	☐	☐
b) heart or blood pressure problems or heart surgery?	☐	☐
c) cancer, diabetes, ulcerative colitis or Crohn's disease?	☐	☐
d) kidney, lung or blood condition?	☐	☐
6. Have you been pregnant in the last 6 months or have you breast-fed in the last 3 months?	☐	☐
7. a) Have you had malaria?	☐	☐
b) In the last 3 years, have you lived or visited an area where malaria is common?	☐	☐
8. In the last 12 months, have you had a tattoo, earpiercing, acupuncture, electrolysis, needle stick injury or graft?	☐	☐
9. Have you ever had yellow jaundice (other than at birth), hepatitis or liver disease?	☐	☐

	Yes	No
10. In the last 12 months, to your knowledge, have you come in close (intimate) contact with someone with yellow jaundice or hepatitis?	☐	☐
11. In the last 12 months, have you received blood, plasma, clotting factors or immune globulin?	☐	☐
12. The following activities put you at risk for AIDS. - if male, having sex with another male, even once - sharing needles or taking street drugs by needle - receiving regular treatment with blood or blood products - accepting money or drugs in exchange for sex - being the sexual partner of someone who has taken part in any of the above activities or who has contracted AIDS or has tested positive for AIDS. Since 1977, have you participated in any of the above activities?	☐	☐
13. In the past 12 months have you: - had sex for which you paid money or drugs?	☐	☐
- had sex with someone who may have participated in high risk activities (sexual background uncertain)?	☐	☐
- had an episode of syphilis or gonorrhea?	☐	☐
14. Have you had an AIDS test elsewhere (other than for donating blood)?	☐	☐
15. The symptoms of AIDS include: - weight loss, night sweats, fever, diarrhea or cough - lumps in the armpits, neck or groin - coloured patches on skin or inside mouth In the last 12 months, have you had any of these symptoms which are continuous and unexplained?	☐	☐

I have read, understood and completed the above questions. The medical history I have given is true. I understand the procedure and any side effects and complications associated with my (whole blood), (plasmapheresis), (cytapheresis) donation. I understand that my blood will be tested for signs of Hepatitis, Syphilis, AIDS and other infectious diseases transmitted by blood and that positive results will be reported to the Public Health Department where required by provincial law. I understand that my blood may not be used because of these tests and that I may be contacted, in confidence, about the results.

Date _____ Donor Name: _____ (Please Print) Donor Signature: _____

cont'd Figure 3

(optional) My results can be released to my Family Doctor whose name is: _____
address _____

438717

Donor accepted ☐ Donor deferred ☐

Comments _____

_____ R.N. Signature _____

438717

BLOOD SAFETY FORM

Some people feel pressured to give blood although it is not safe for their blood to be used for transfusion.

- If none of the risk factors listed below apply to you, you must PLACE A "USE MY BLOOD" STICKER ON THE SPACE BELOW.
- If any of the risk factors listed below apply to you, PLACE A "DON'T USE MY BLOOD" STICKER ON THE SPACE BELOW.

Your information will be kept confidential.

High Risk for AIDS

1. If male, having sex with another man, even once since 1977.
2. Sharing needles or taking street drugs by needle since 1977.
3. Receiving regular treatment with blood or blood products since 1977.
4. Having sex in exchange for money or drugs, since 1977.
5. Having a test that confirms that you have been exposed to the AIDS virus (or have the AIDS virus now).
6. Being the sexual partner of someone who has taken part in any of the above activities, or who has contracted AIDS or has tested positive for AIDS.

PLACE BAR-CODED LABEL HERE

Figure 4 Distribution of HIV negative and HIV positive LOD readings. (This figure is from [15].)

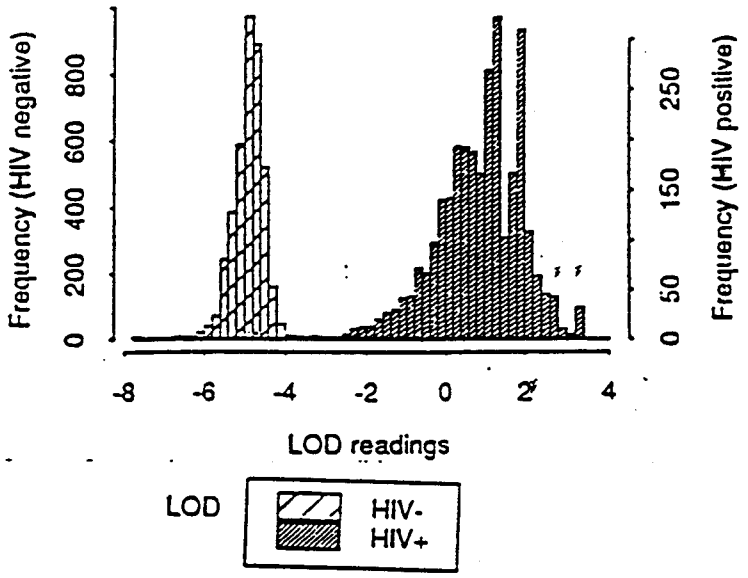


Figure 5a Expected number of sample classified by a single test versus group size for MS when prevalence rate is 0.00009 and probability of a false positive outcome is 0.031.

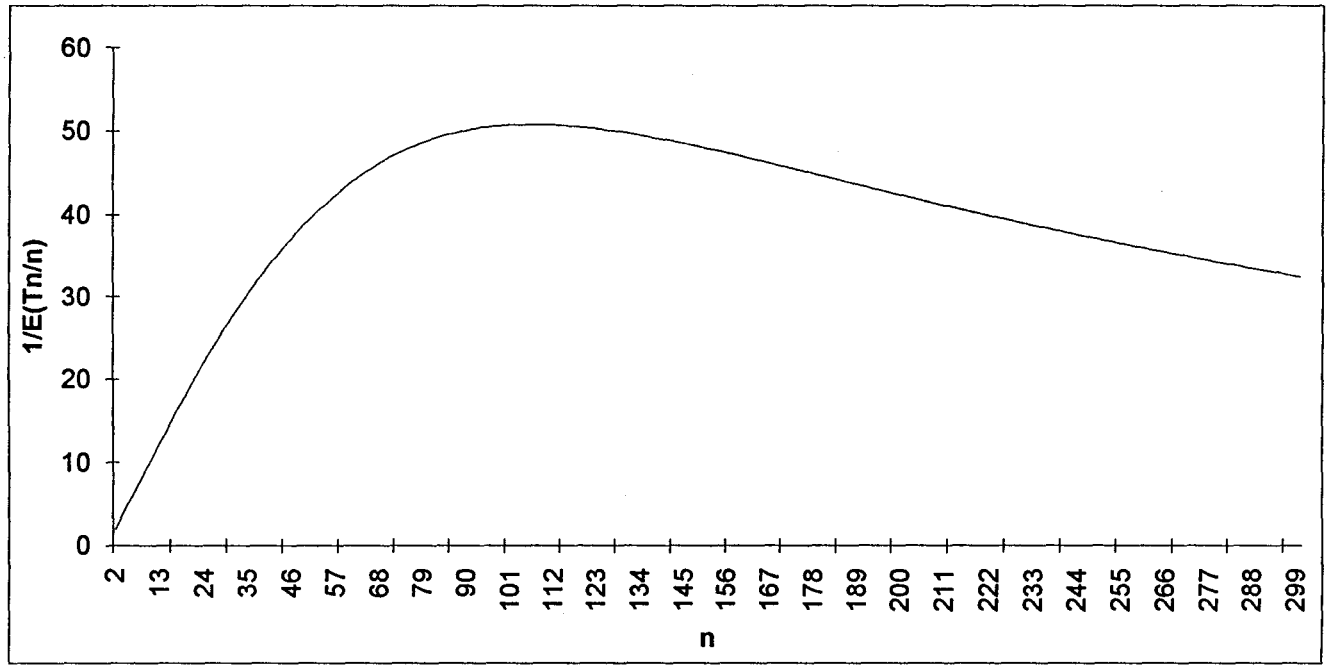


Figure 5b Expected number of sample classified by a single test versus group size for MS when prevalence rate is 0.01 and probability of a false positive outcome is 0.0005.

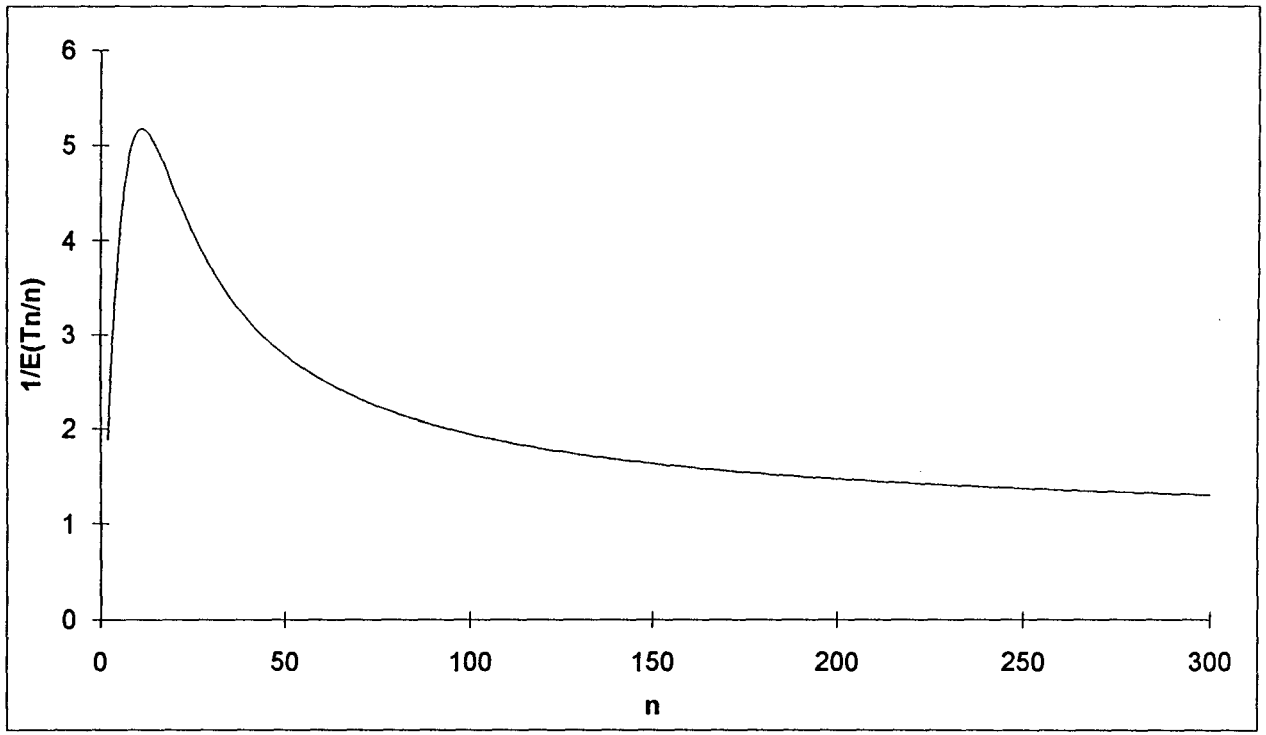


Figure 5c Expected number of sample classified by a single test versus group size for MS when prevalence rate is 0.00009 and probability of a false positive outcome is 0.98.

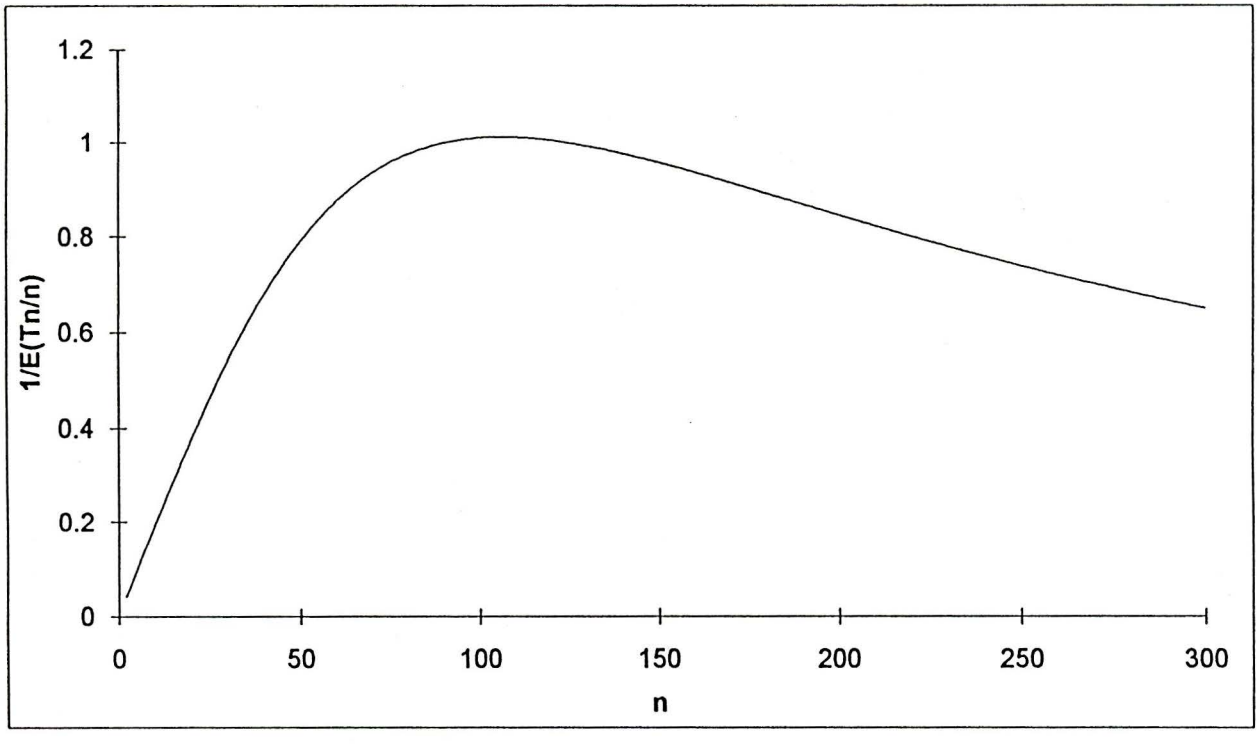


Figure 5d Expected number of sample classified by a single test versus group size for MS when prevalence rate is 0.1 and probability of a false positive outcome is 0.00001.

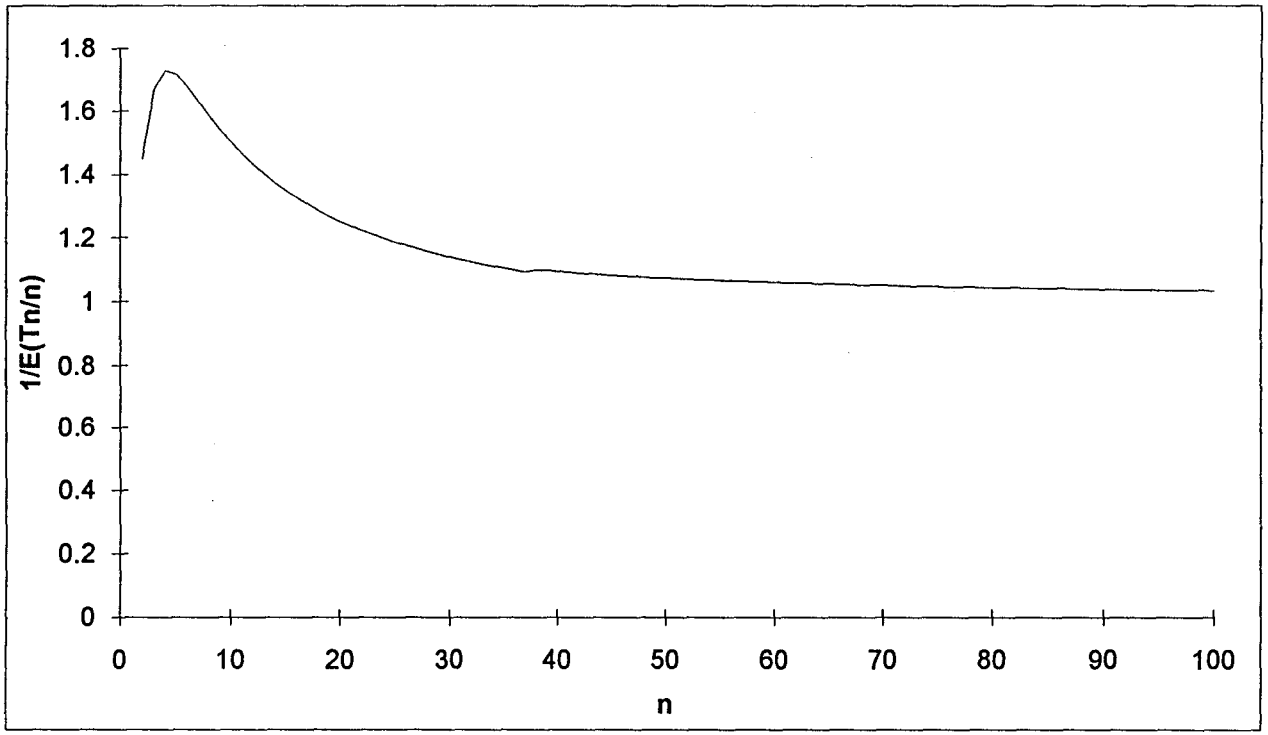


Figure 6a Expected number of sample classified by a single test versus group size for MD when prevalence rate is 0.00009 and probability of a false positive outcome is 0.031.

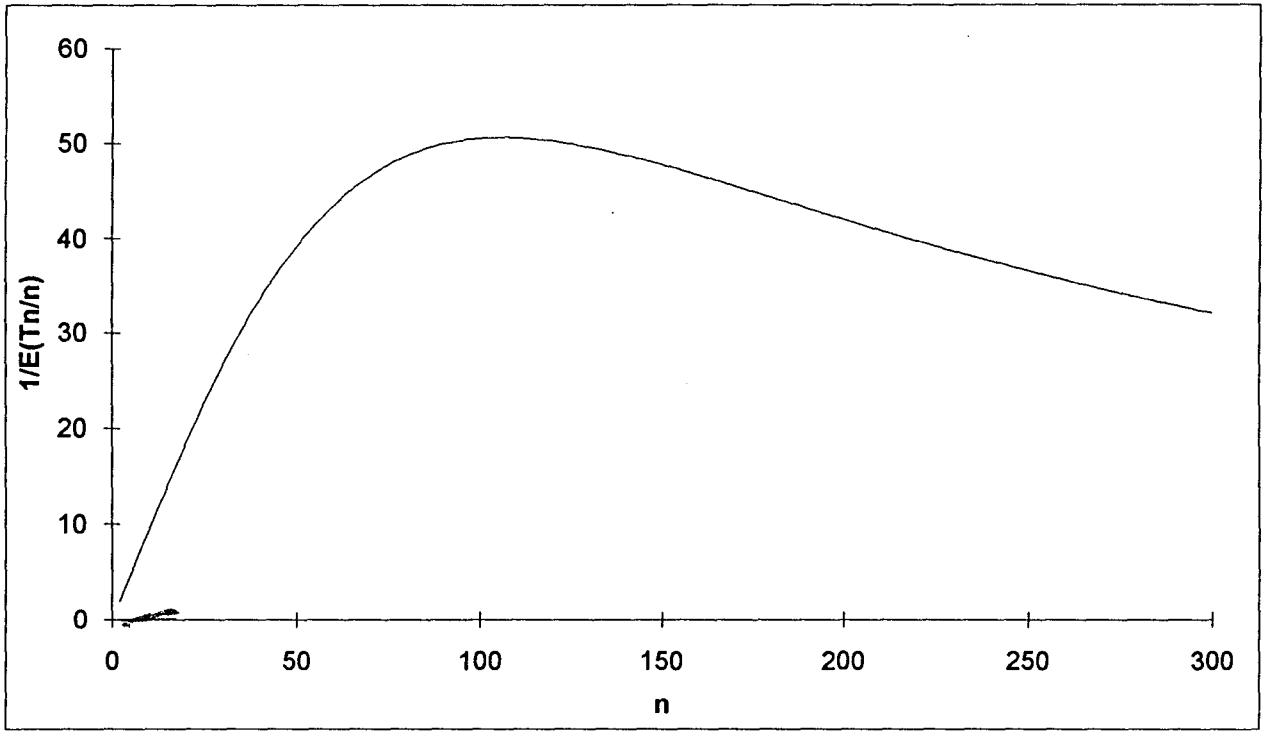


Figure 6b Expected number of sample classified by a single test versus group size for MD when prevalence rate is 0.01 and probability of a false positive outcome is 0.0005.

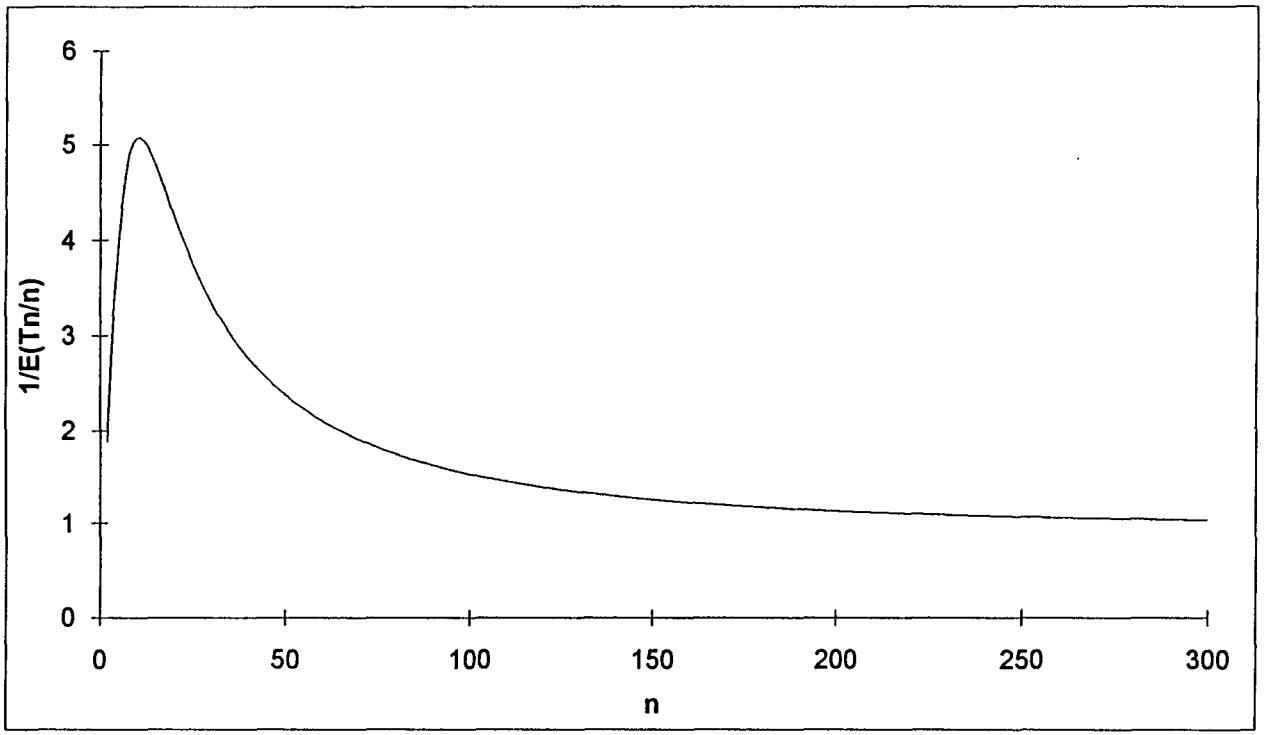


Figure 6c Expected number of sample classified by a single test versus group size for MD when prevalence rate is 0.00009 and probability of a false positive outcome is 0.98.

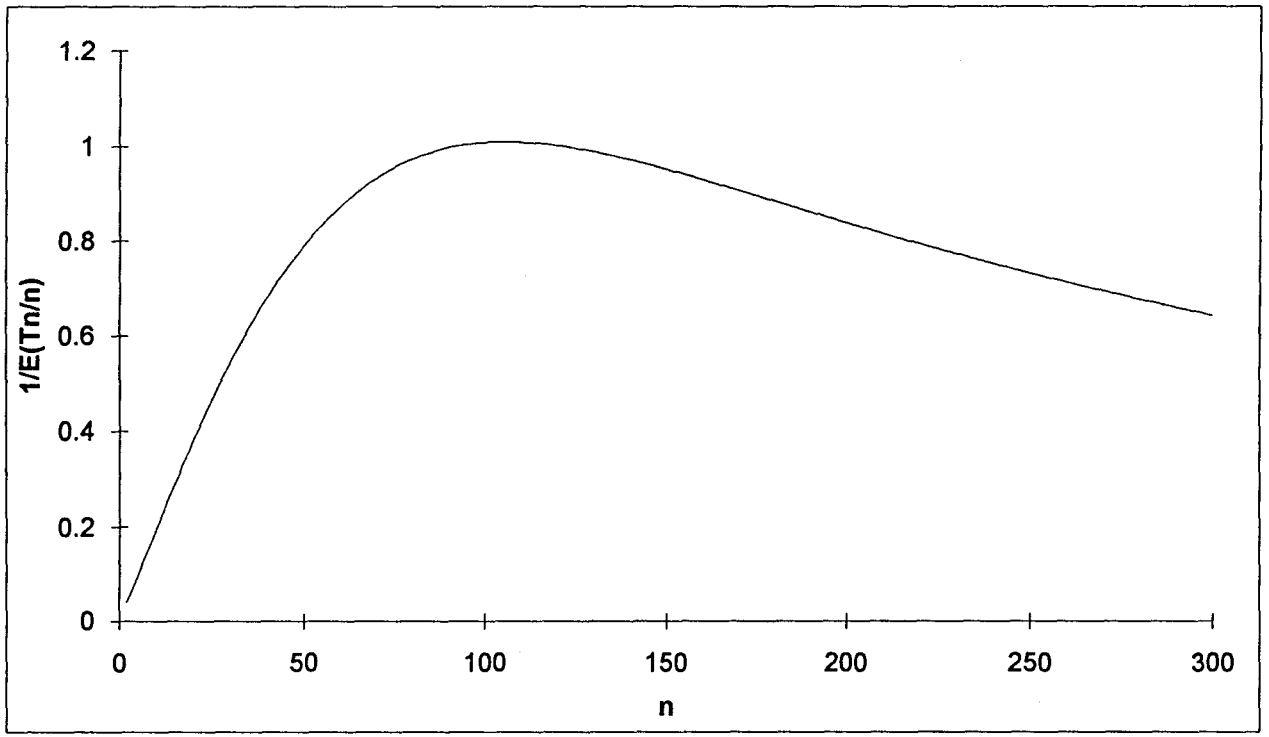


Figure 6d Expected number of sample classified by a single test versus group size for MD when prevalence rate is 0.1 and probability of a false positive outcome is 0.00001.

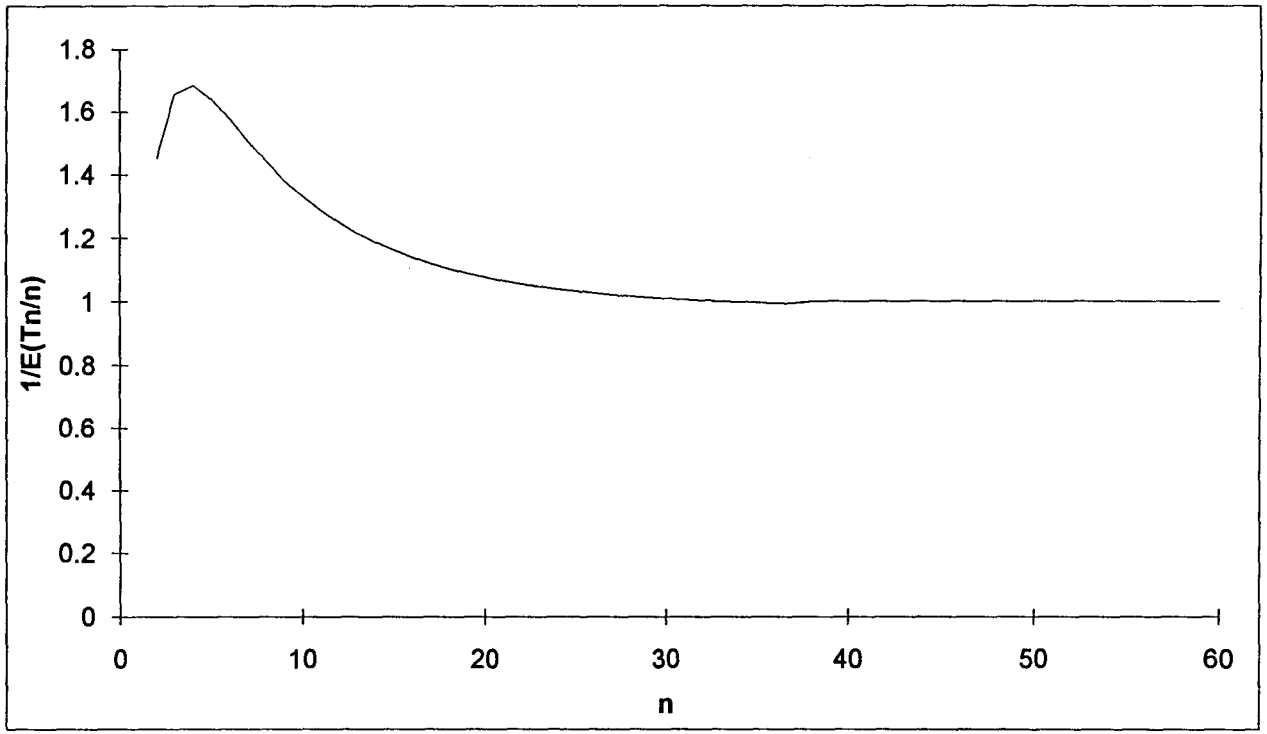


Figure 7a Expected number of samples classified by a single test which corresponds to the optimal group size versus probability of a false positive outcome for MS when prevalence rate is 0.05.

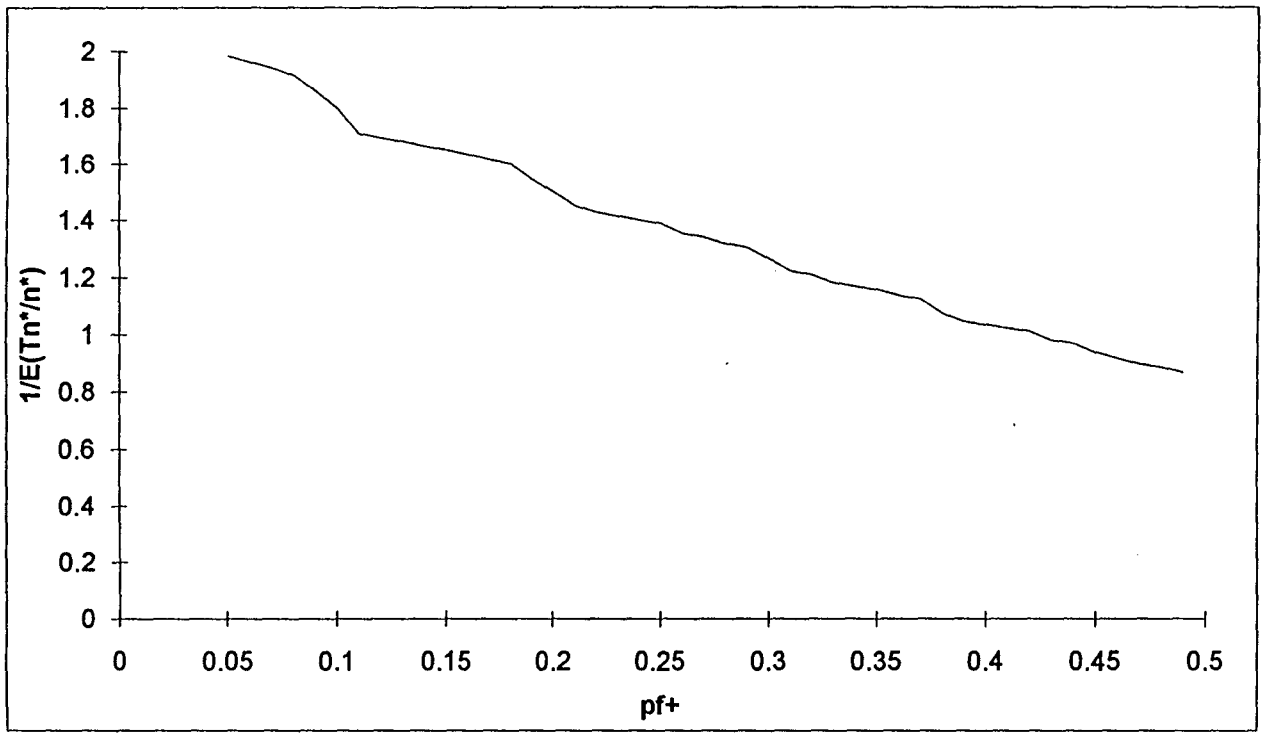


Figure 7b Expected number of samples classified by a single test which corresponds to the optimal group size versus prevalence rate for MS when probability of a false positive outcome is 0.5.

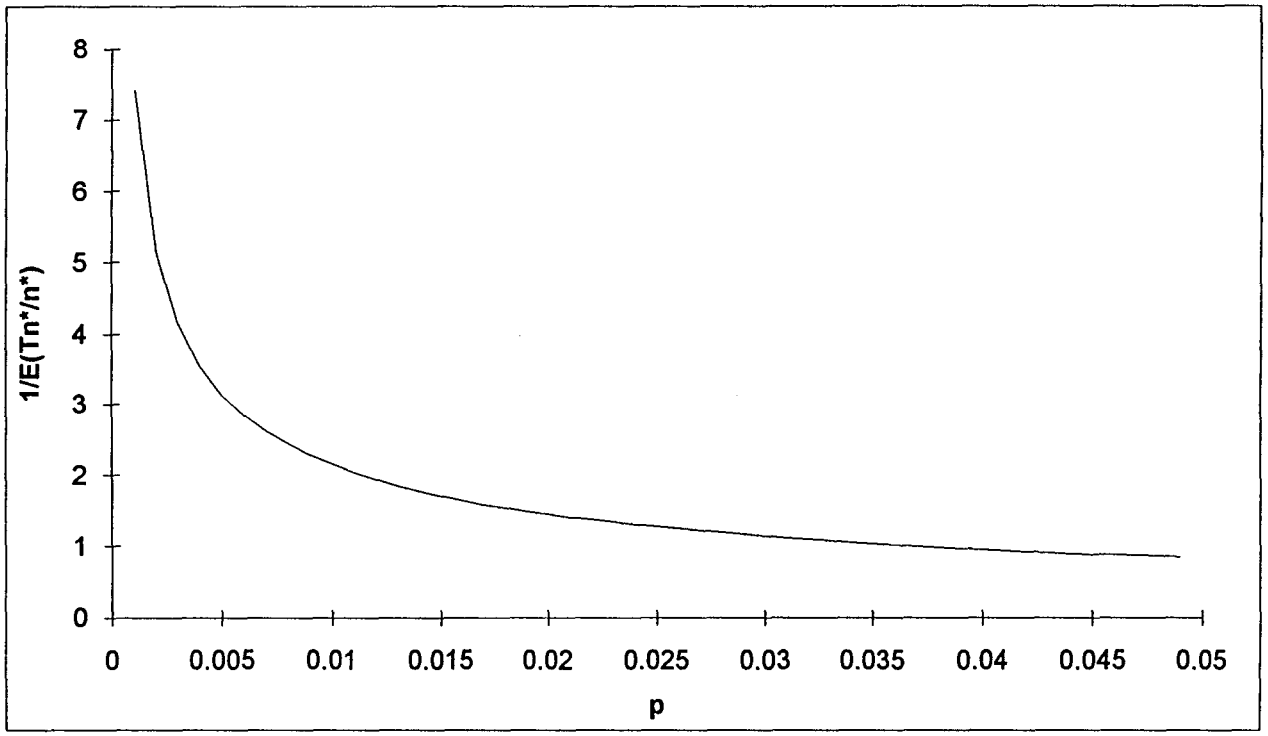


Figure 7c Expected number of samples classified by a single test which corresponds to the optimal group size versus probability of a false positive outcome for MS when prevalence rate is 0.001.

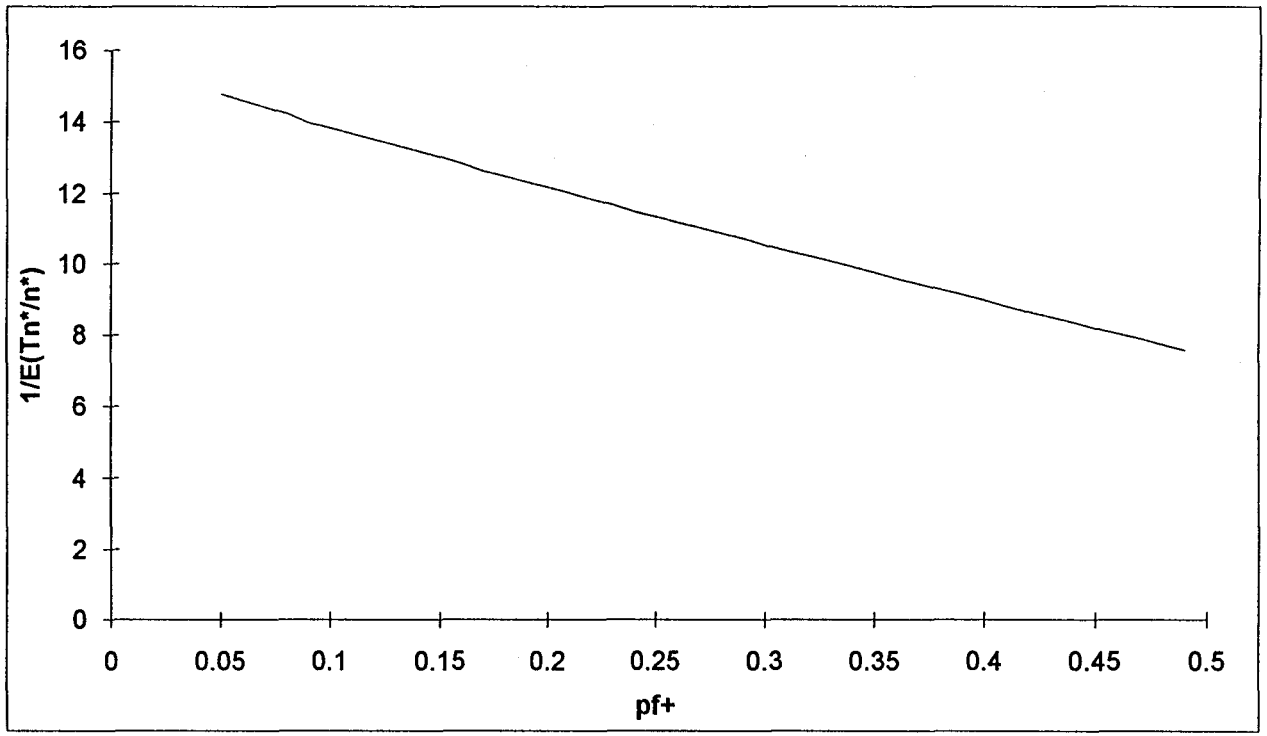


Figure 7d Expected number of samples classified by a single test which corresponds to the optimal group size versus prevalence rate for MS when probability of a false positive outcome is 0.05.

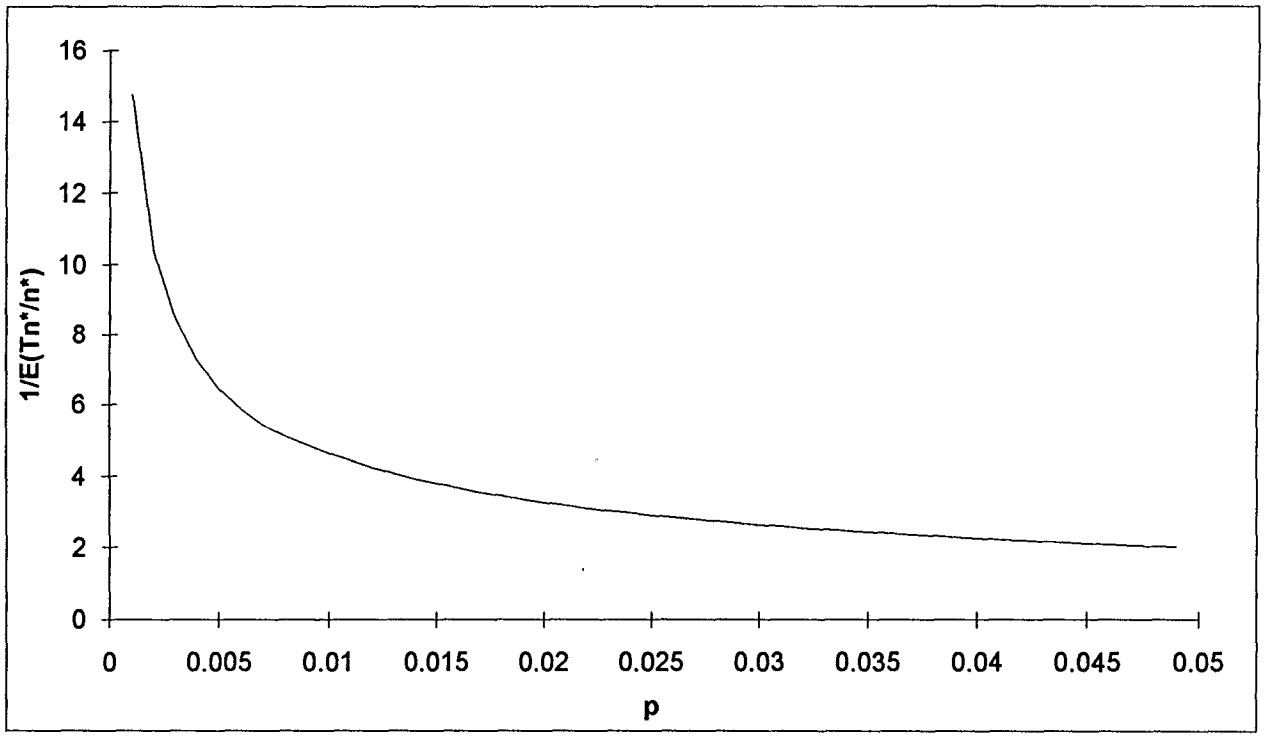


Figure 8a Expected number of samples classified by a single test which corresponds to the optimal group size versus probability of a false positive outcome for MD when prevalence rate is 0.05.

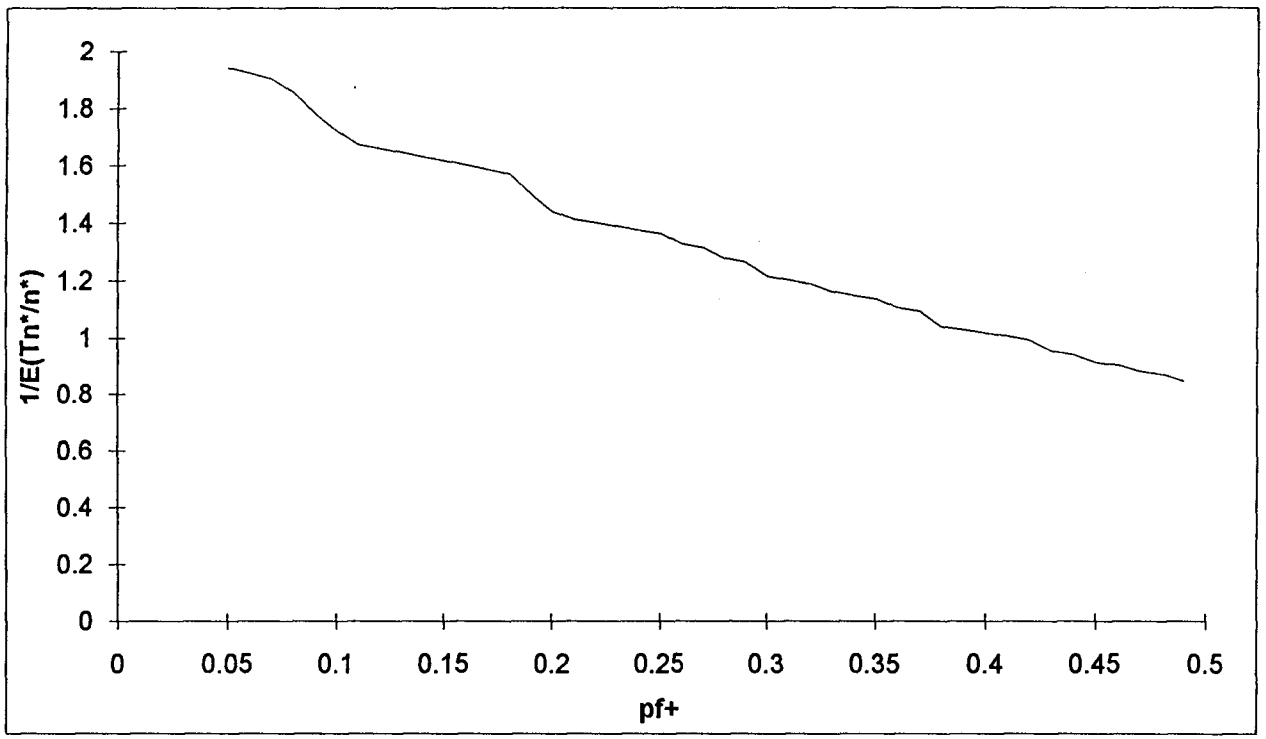


Figure 8b Expected number of samples classified by a single test which corresponds to the optimal group size versus prevalence rate for MD when probability of a false positive outcome is 0.5.

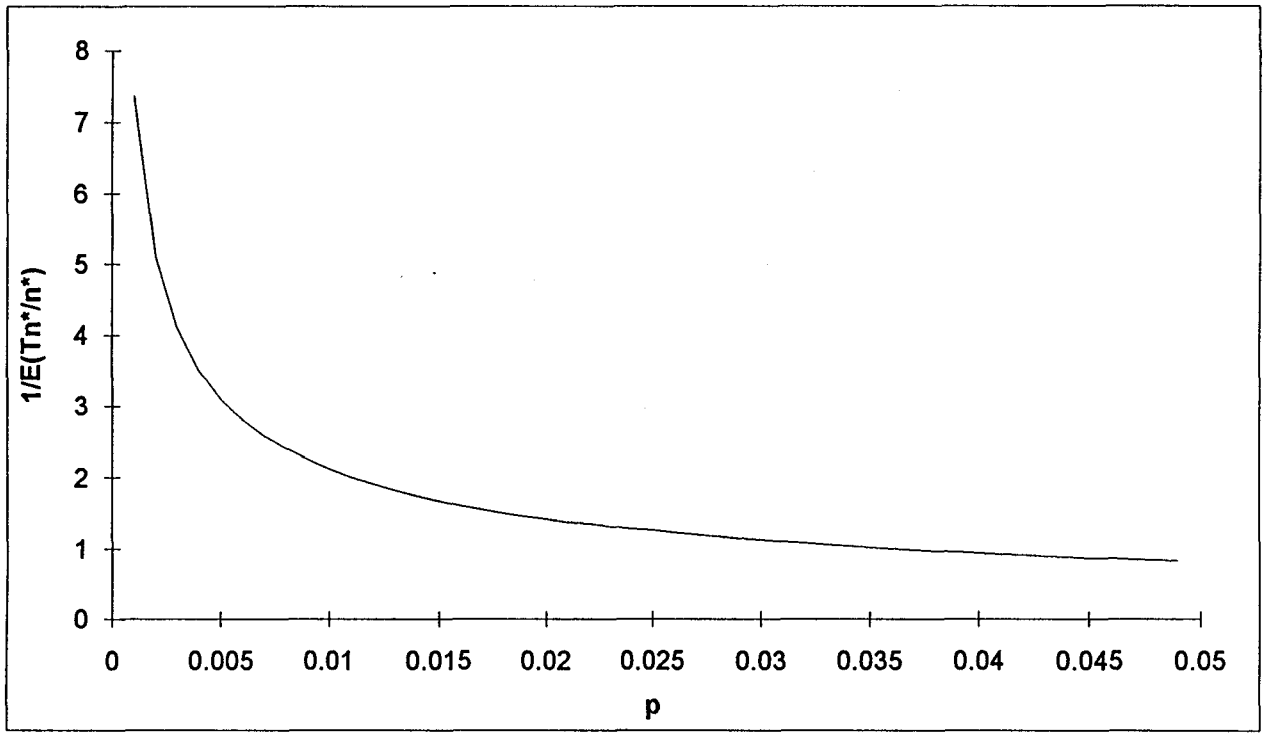


Figure 8c Expected number of samples classified by a single test which corresponds to the optimal group size versus probability of a false positive outcome for MD when prevalence rate is 0.001.

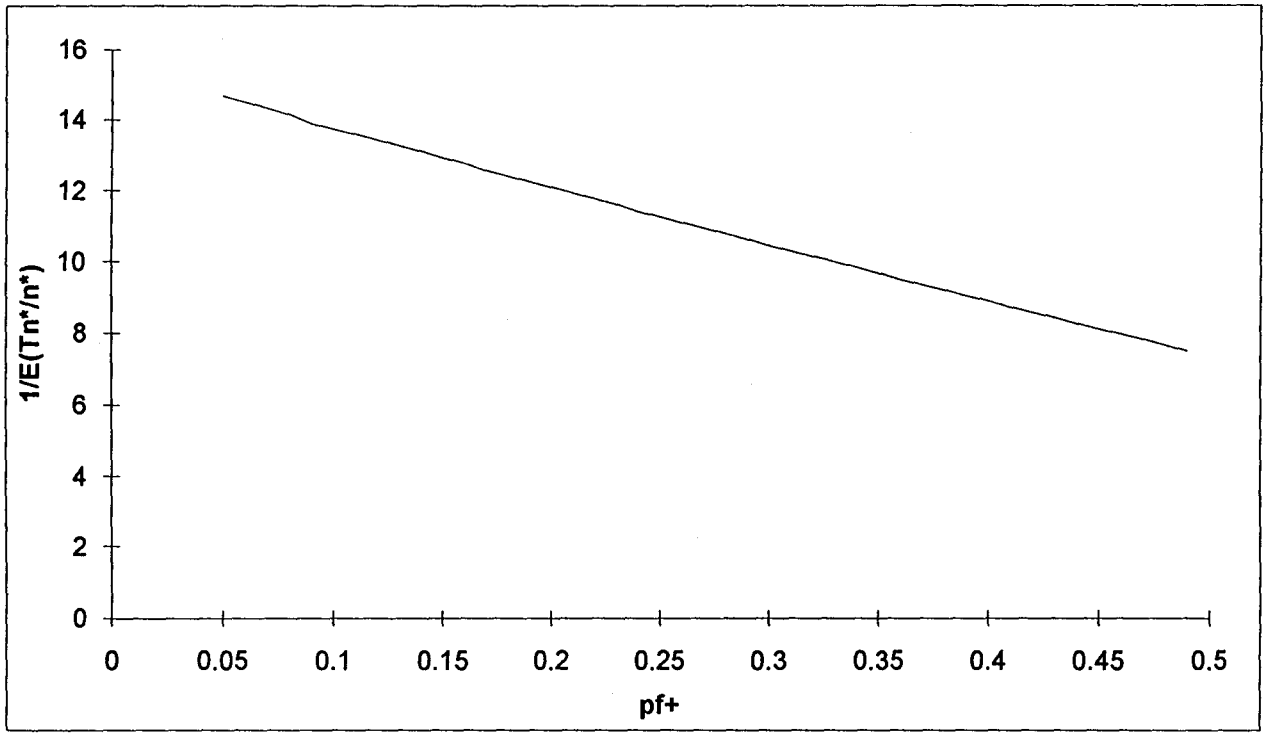


Figure 8d Expected number of samples classified by a single test which corresponds to the optimal group size versus prevalence rate for MD when probability of a false positive outcome is 0.05.

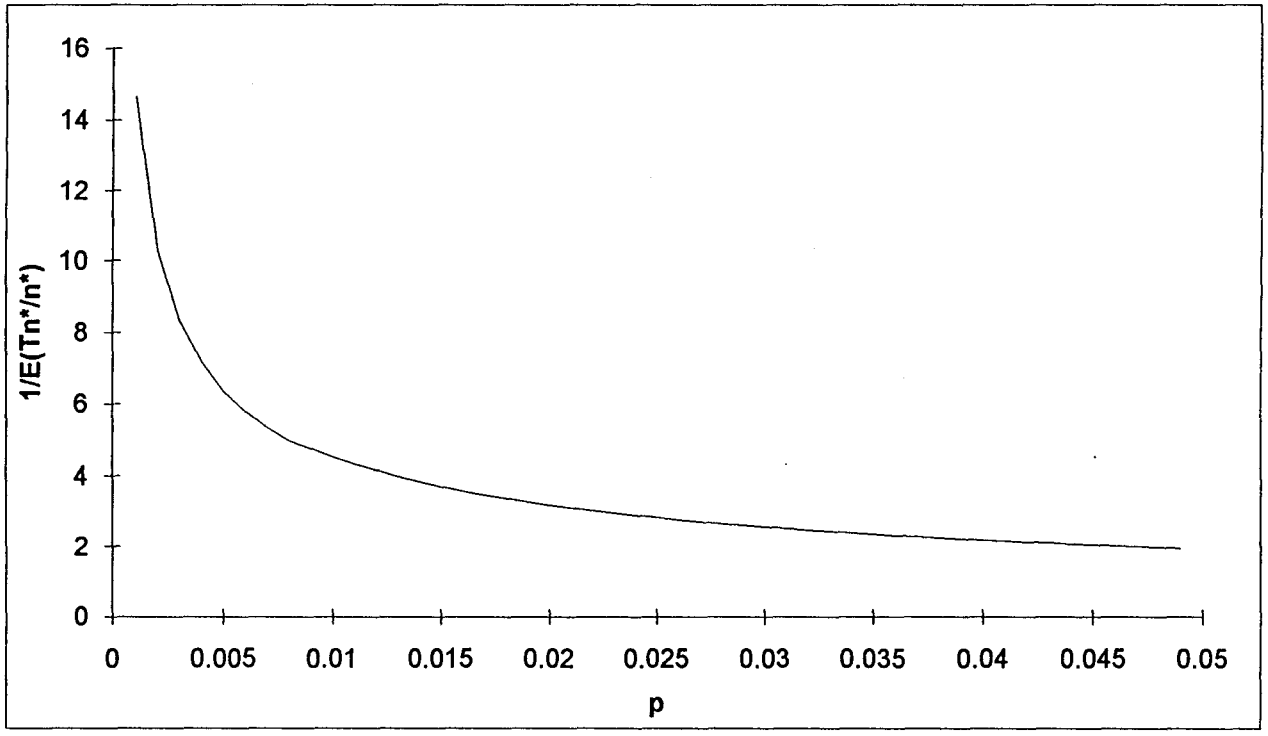


Figure 9a Number of positive tests necessary to classify a group of size 60 as positive versus probability of a false positive outcome when prevalence rate is 0.00009.

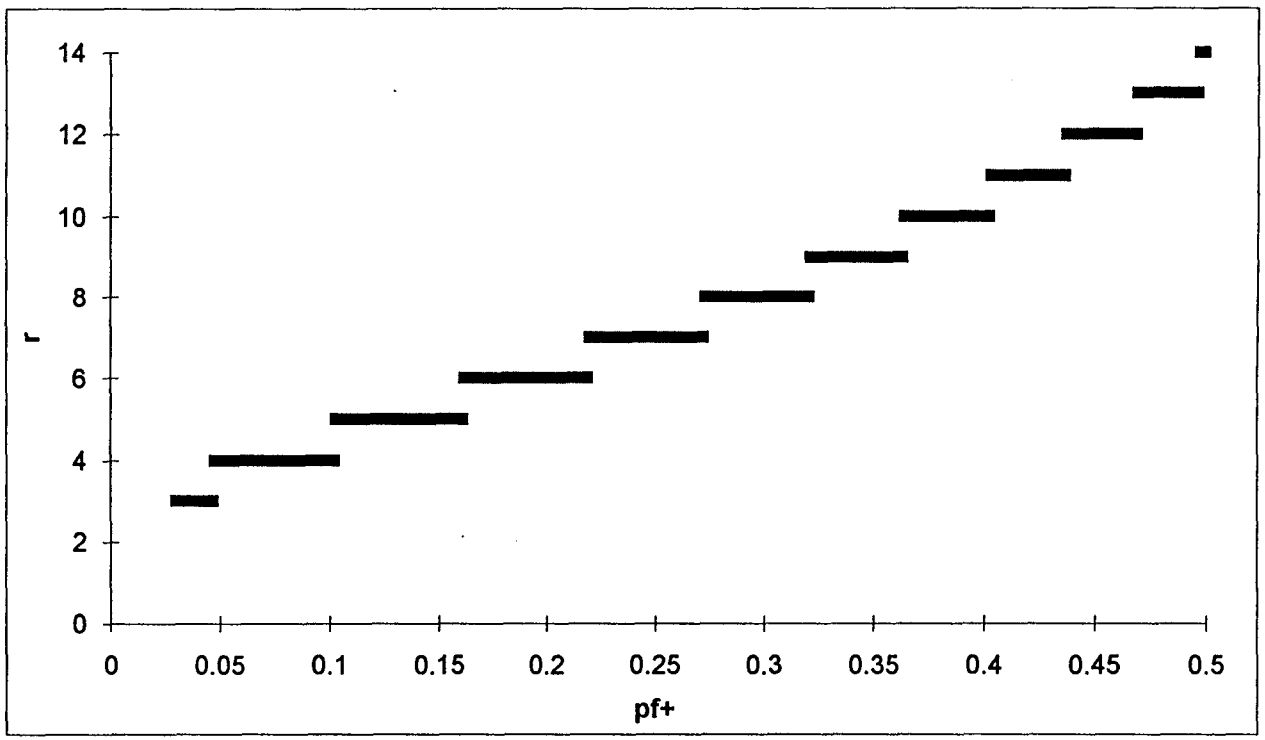


Figure 9b Number of positive tests necessary to classify a group of size 60 as positive versus prevalence rate when probability of a false positive outcome is 0.3.

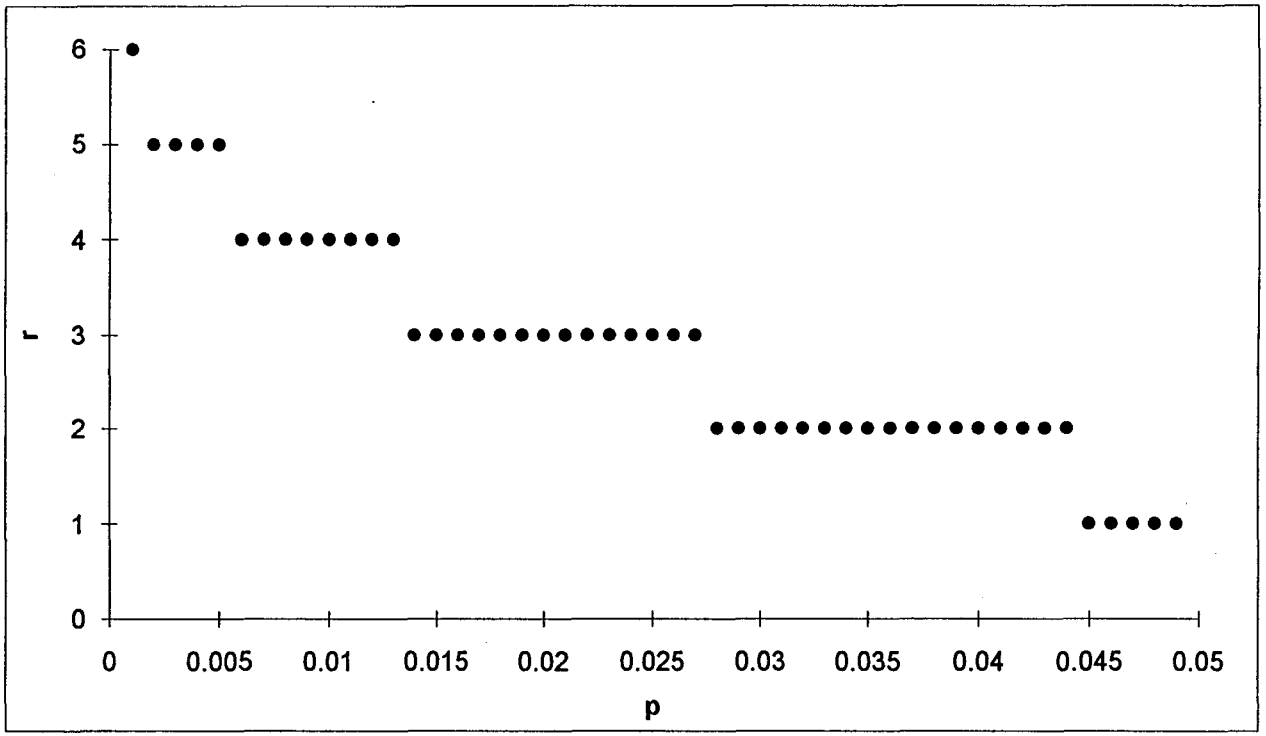


Figure 10a Number of positive tests necessary to make a positive classification versus group size when prevalence rate is 0.00009 and probability of a false positive outcome is 0.031.

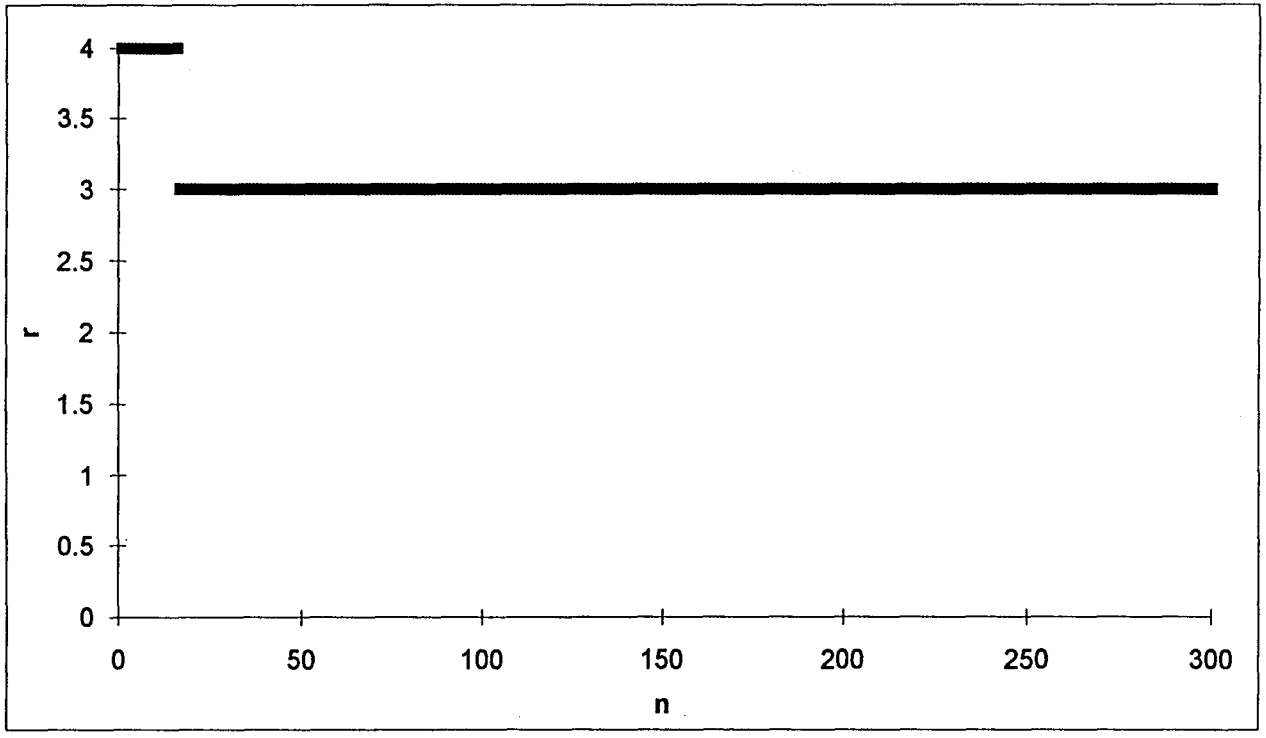


Figure 10b Number of positive tests necessary to make a positive classification versus group size when prevalence rate is 0.00009 and probability of a false positive outcome is 0.8.

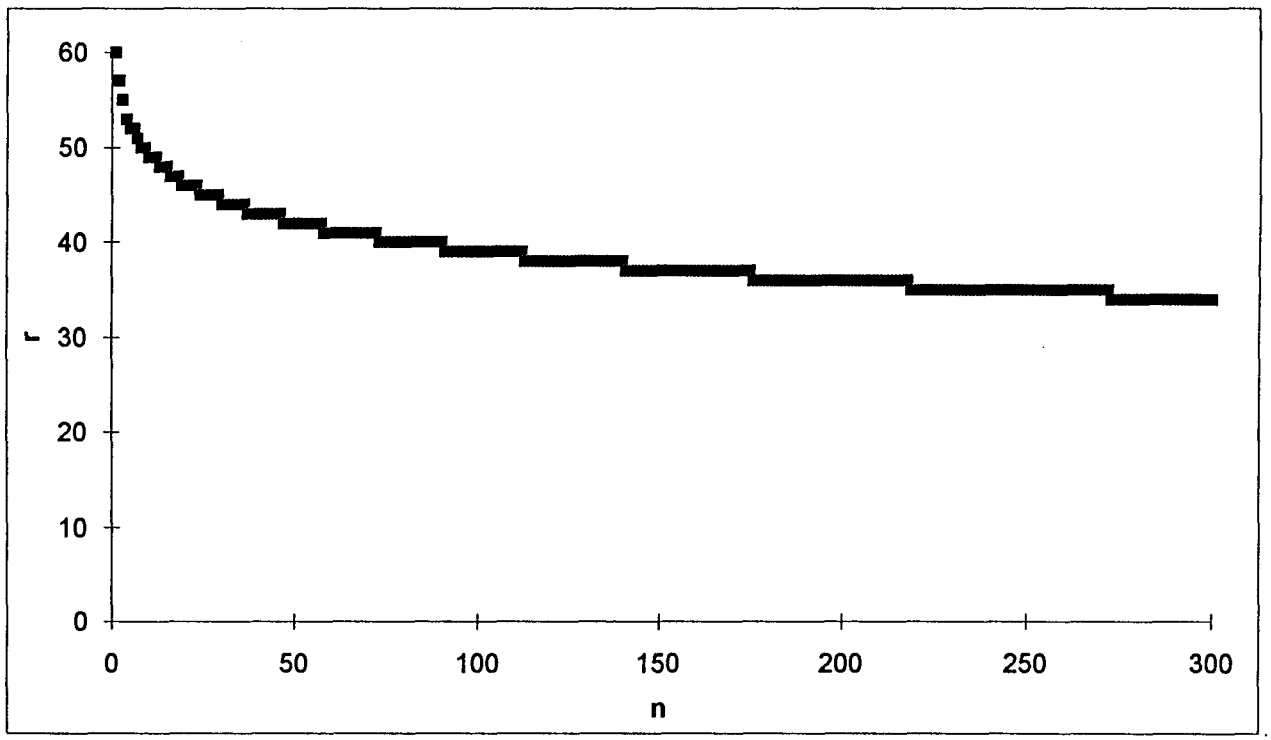


Figure 11a Optimal group size versus probability of a false positive outcome for MS when prevalence rate is 0.05.

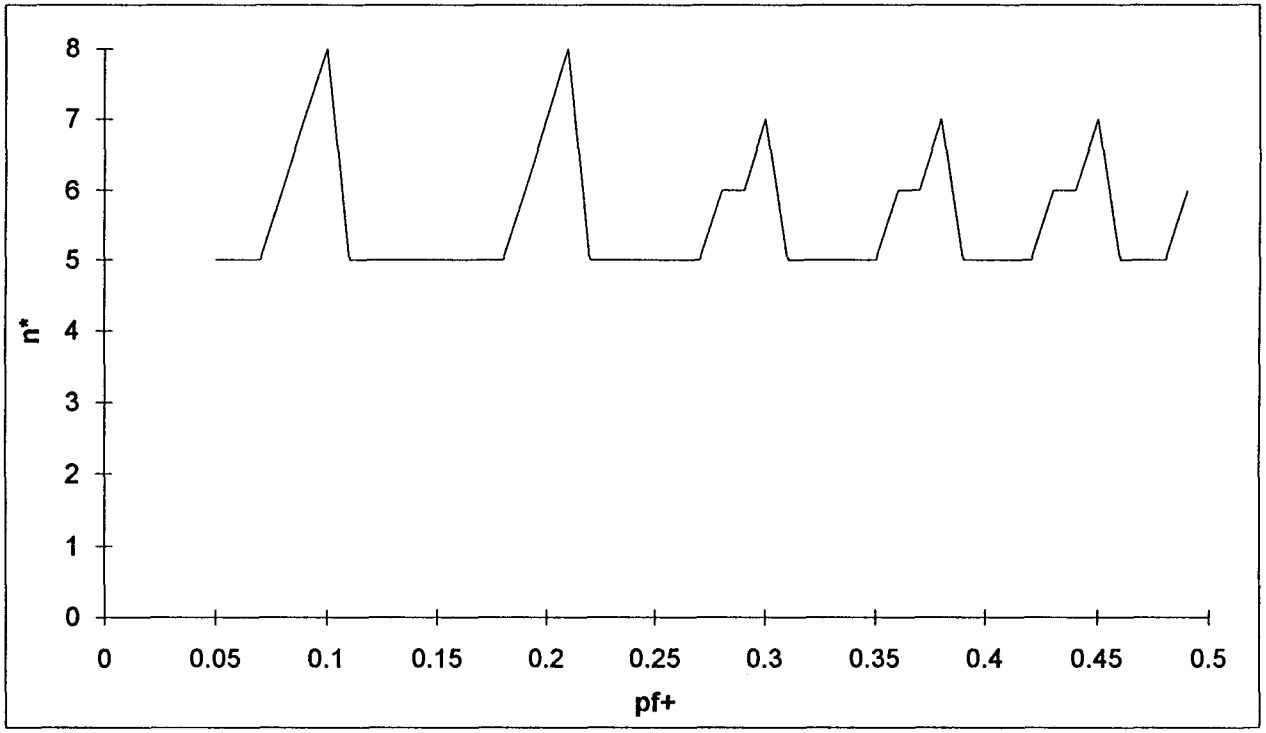


Figure 11b Optimal group size versus prevalence rate for MS when probability of a false positive outcome is 0.5.

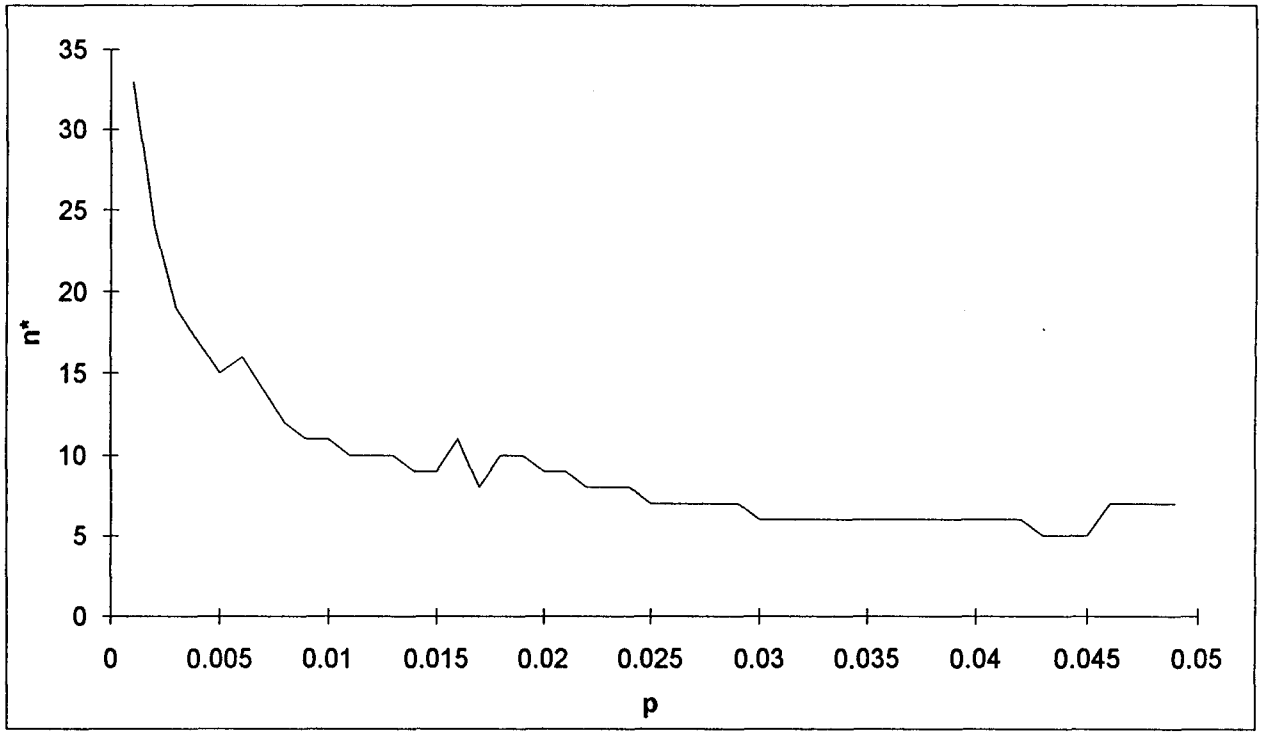


Figure 11c Optimal group size versus probability of a false positive outcome for MD when prevalence rate is 0.05.

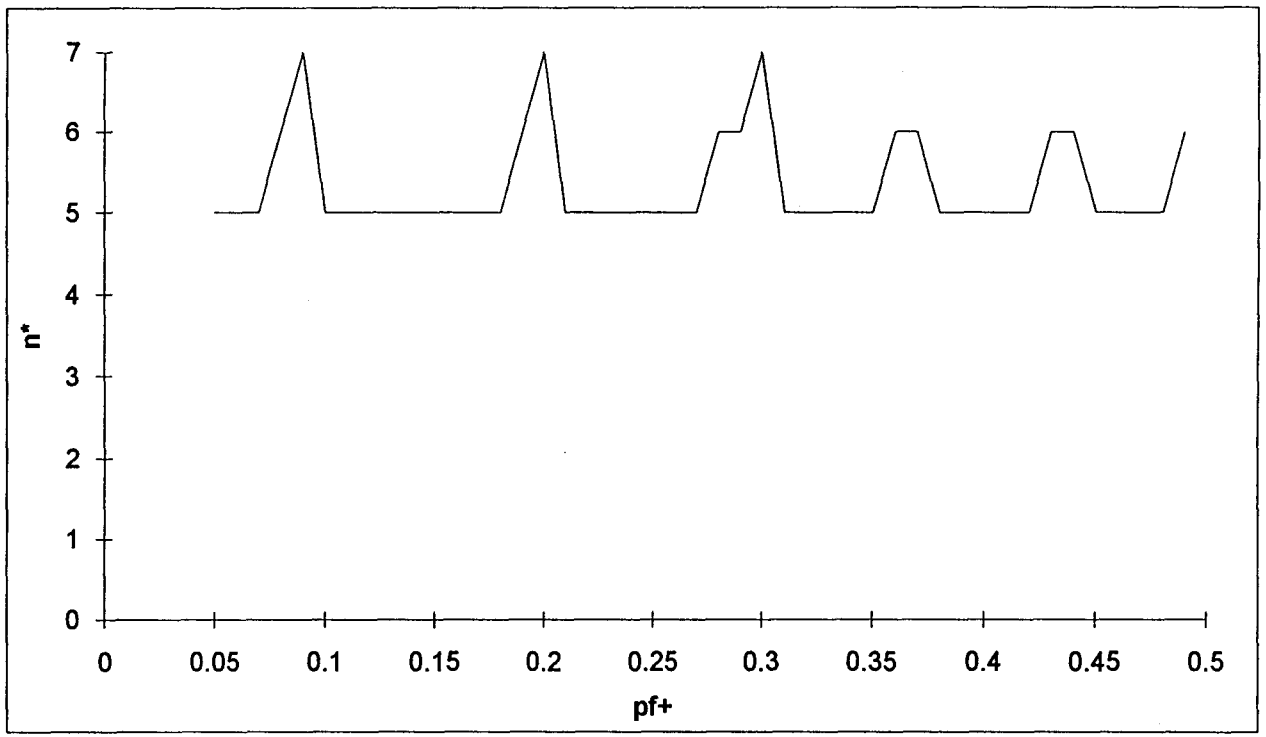


Figure 11d Optimal group size versus prevalence rate for MD when probability of a false positive outcome is 0.5.

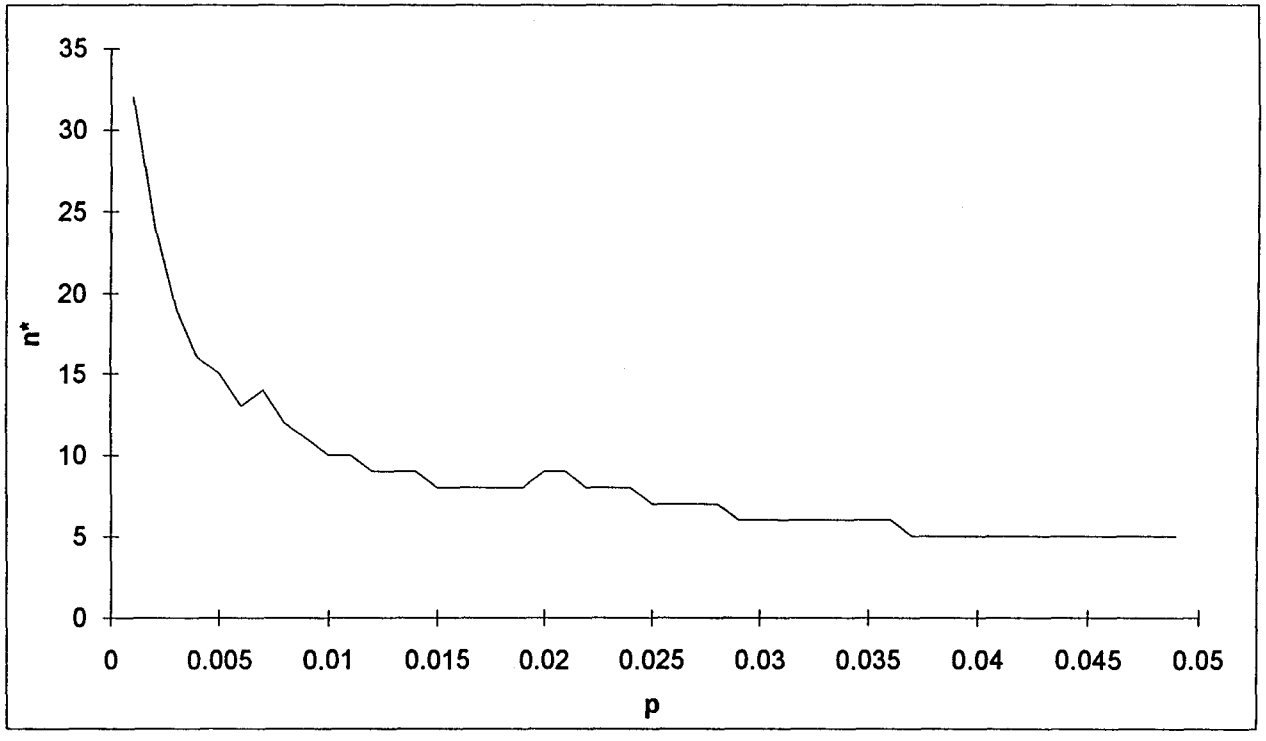


Figure 12a Coefficient of variation of the number of tests per sample versus sample size for MS when prevalence rate is 0.00009 and probability of a false positive outcome is 0.031.

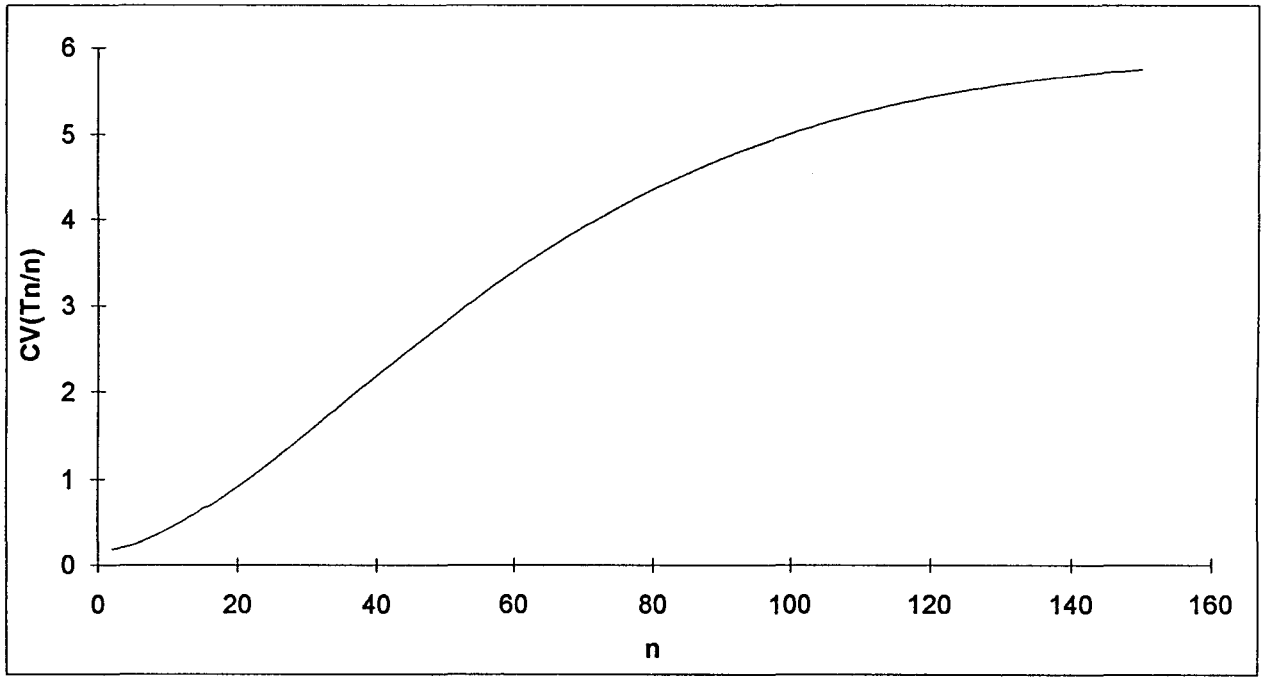


Figure 12b Coefficient of variation of the number of tests per sample versus sample size for MS when prevalence rate is 0.00009 and probability of a false positive outcome is 0.98.

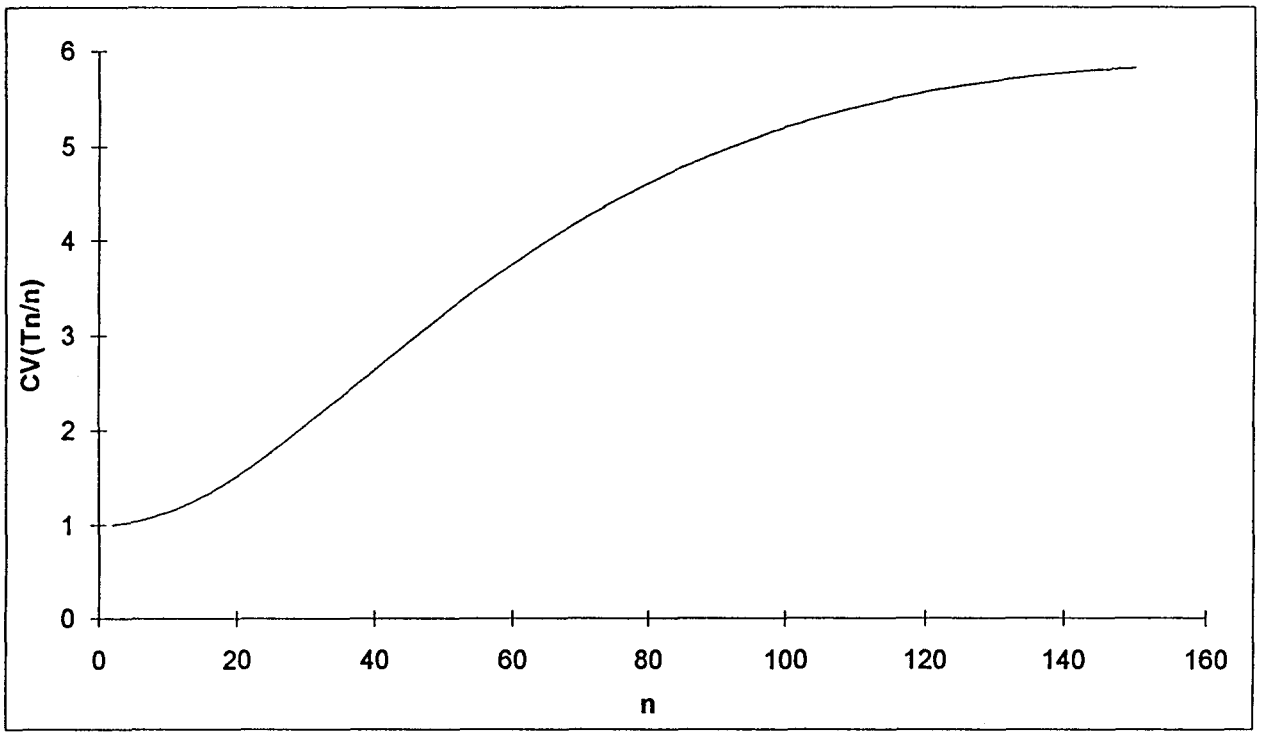


Figure 12c Coefficient of variation of the number of tests per sample versus sample size for MD when prevalence rate is 0.00009 and probability of a false positive outcome is 0.031.

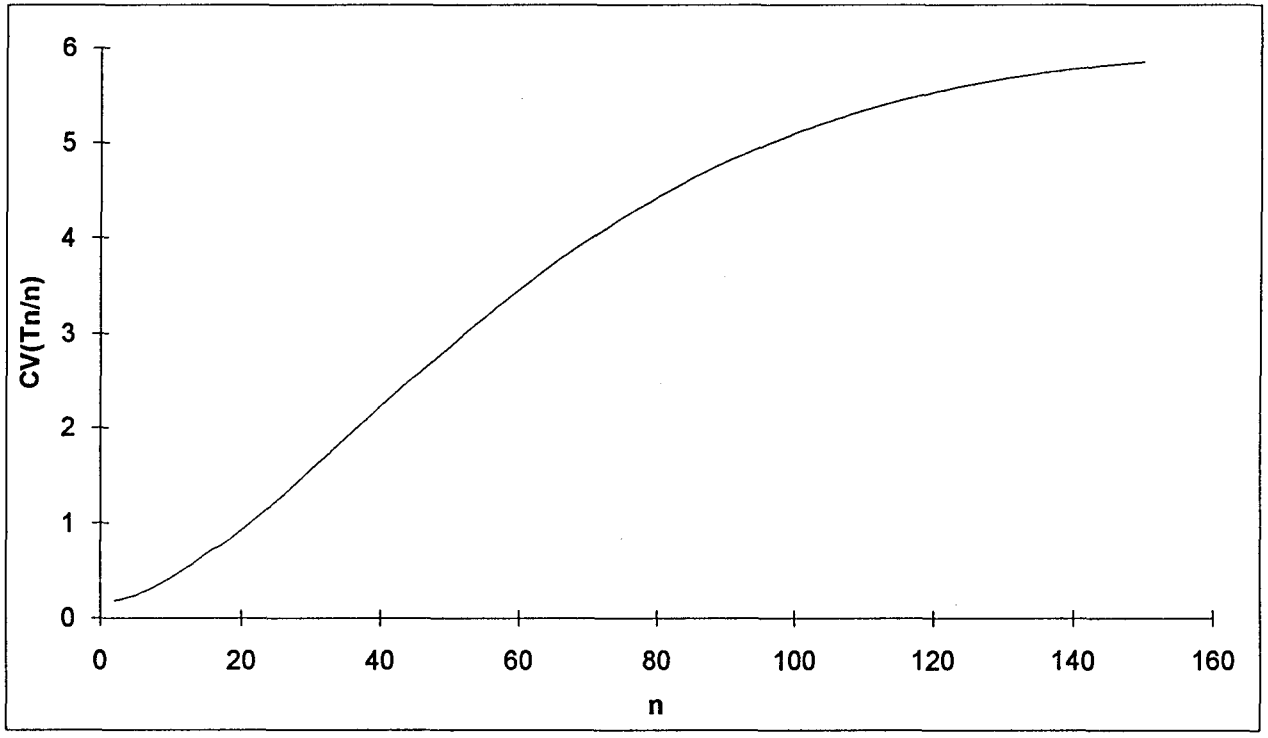
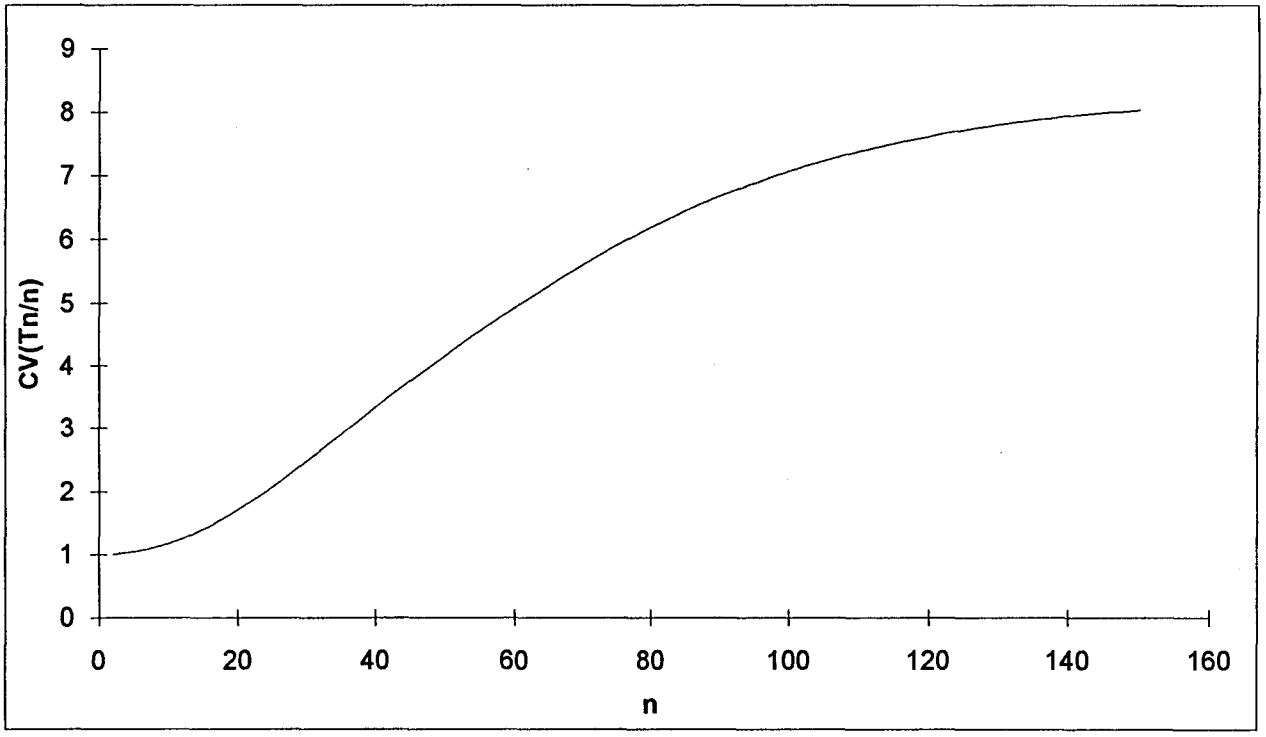


Figure 12d Coefficient of variation of the number of tests per sample versus sample size for MD when prevalence rate is 0.00009 and probability of a false positive outcome is 0.98.



APPENDIX C

Programs

NOTE:
- All programs are written in Borland C++ (Version 3.1 by Borland International Inc.).
- Comments within programs are written in italics. Other comments are written in italics and are surrounded by asterisks.
- Expressions *A,B,C,D,F,H,P,An,Bn,Hn,S,S1,S2* and *S3* are defined on page 23. Expression for *r* is stated on page 29 and *r1* is this expression for *n*=1. Definitions of *r* and *r1* are given on page 21. Random variable T_n is defined on page 22.

Program 1 Calculates expected number of tests per sample and corresponding variance for MS.

```
#include        <stdio.h>
#include        <math.h>
#define         ALFA    .98
#define         N_LIMIT 600

double  p,pf,r,r1;
double  A,B,C,D,F,H,P,An,Bn,Hn,S,S1,S2,S3,V;

double  sum1(int n);
double  sum2(int n);
double  get_t(int n);
double  get_m(int n);
double  tn[N_LIMIT];
double  mn[N_LIMIT];

main()
{
int     n;
double  Tn,Mn;
FILE    *fp;
     fp = fopen("Drive:Filename", "w");
     p=Prevalence Rate;
     pf=Probability of a False Positive Outcome;
        for(n = 2; n <= N_LIMIT; n++)
           {
           Tn = get_t(n);            *E(Tn)*
           Mn = get_m(n);            *E(Tn^2)*
           tn[n] = Tn;
           mn[n] = Mn;
           V=Mn/pow((double)n,2.0)-
             pow(Tn/n,2.0);          *Var(Tn/n)*
           fprintf(fp, "n:%d E(Tn/n):%.10f
                   1/(E(Tn/n)):%.5f
                   var:%.5f cv:%.5f\n",
                   n,Tn/n,n/Tn,V,
```

```
                    sqrt(V)/(Tn/n));
            }
        fclose(fp);
        return(0);

}
double  get_t(int n)
{
double  res1;
r=ceil(log((1-ALFA)*(1-pow(1-p,(double)n))
  /(ALFA*pow(1-p,(double)n)))/log(pf));
r1=ceil(log(p*(1-ALFA)/((1-p)*ALFA))/log(pf));
A=1-(r1+1)*pow(pf,r1)+r1*pow(pf,(double)(r1+1));
C=p+(1-p)*pow(pf,(double)r1);
D=1+pf;
F=1-pf;
An=1-(r+1)*pow(pf,(double)r)+r*pow(pf,(double)(r+1));
Bn=pow(pf,(double)r);
P=r1*(1-pow(1-C,(double)n))+A*(1-p)*(1+pow(1-C,(double)
  (n-1))*(C*(1-n)-1))/(F*C)+A*(1-p)*n*
  pow(1-C,(double)(n-1))/F;
res1 =sum1(n)+(r+P)*(1-(1-Bn)*pow(1-p,(double)n))+
          An*pow(1-p,(double)n)/F;
return(res1);
}
double get_m(int n)
{
double res2;
r=ceil(log((1-ALFA)*(1-pow(1-p,(double)n))
  /(ALFA*pow(1-p,(double)n)))/log(pf));
r1=ceil(log(p*(1-ALFA)/((1-p)*ALFA))/log(pf));
A=1-(r1+1)*pow(pf,(double)r1)+r1*
  pow(pf,(double)(r1+1));
C=p+(1-p)*pow(pf,(double)r1);
D=1+pf;
F=1-pf;
B=pow(pf,(double)r1);
H=(D*A-r1*(r1+1)*B*pow(F,2.0))*(1-p)/pow(F,2.0);
An=1-(r+1)*pow(pf,(double)r)+r*pow(pf,(double)(r+1));
Bn=pow(pf,(double)r);
Hn=(D*An-r*(r+1)*Bn*pow(F,2.0))*pow(1-p,(double)n)/
   pow(F,2.0);
P=r1*(1-pow(1-C,(double)n))+A*(1-p)*(1+pow(1-C,(double)
  (n-1))*(C*(1-n)-1))/(F*C)+A*(1-p)*n*pow(1-C,(double)
  (n-1))/F;
S1=H/(1-C)+2*r1*A*(1-p)/(F*(1-C));
S2=1-(n+1)*pow(1-C,(double)n)+n*pow(1-C,(double)(n+1));
S3=pow(A*(1-p)/(F*(1-C)),2.0);
S=C*r1*(1-r1)+H*n*pow(1-C,(double)(n-1))+(n-1)*n*S3*
```

```
  pow(1-C,(double)n)+(1-pow(1-C,(double)n))*
  (2*S3-S1+pow(r1,2.0))+S3*(S2*(2-C)-n*(n+1)*
  pow(1-C,(double)n)*pow(C,2.0))/pow(C,2.0)+
  S2*(S1-3*S3)/C;

res2=Hn+(1-pow(1-p,(double)n)*(1-Bn))*
     (pow(r,2.0)+2*r*P+S)+sum2(n);
return (res2);
}
double  sum1(int n)
{
double  t;
int     j;
     t = 0;
     tn[1] =r1*C+(1-p)*A/F;
     for(j = 1; j <= n-1; j++)
t=t + (1-(1-Bn)*pow(1-p,(double)n))*C*
  pow(1-C,(double)(j-1))*tn[n-j];
return(t);
}
double sum2(int n)
{
double x;
int    y;
       x=0;
       tn[1] =r1*C+(1-p)*A/F;
       mn[1] =pow(r1,2.0)*C+H;
       for(y=1;y<=n-1;y++)
x=x+2*(1-pow(1-p,(double)n)*(1-Bn))*(r+r1+(y-1)*A*
  (1-p)/(F*(1-C)))*C*pow(1-C,(double)(y-1))*tn[n-y]+
  (1-pow(1-p,(double)n)*(1-Bn))*C*
  pow(1-C,(double)(y-1))*mn[n-y];
return (x);
}
```

Program 2 Calculates minimum expected number of tests per sample and corresponding variance and group size for MS. (In this program parameters are set to the following values:
p=0.001...0.02, pf_+=0.05...0.5, n=2...150.)

```
#include        <stdio.h>
#include        <math.h>
#define         ALFA     .98
#define         N_LIMIT 150
#define         P_LIMIT  0.02
#define         PF_LIMIT 0.5

double  p,pf,r,r1;
```

```
double  A,B,C,D,F,H,P,An,Bn,Hn,S,S1,S2,S3;

double  sum1(int n);
double  sum2(int n);
double  get_t(int n);
double  get_m(int n);
double  tn[N_LIMIT];
double  mn[N_LIMIT];

main()
{
int     n,i,k;
double  Tn,Mn,min,smin,V;
FILE    *fp;
     fp = fopen("Drive:Filename", "w");
     for (p=0.001;p<=P_LIMIT;p=p+0.001)
           for(pf=0.05;pf<=PF_LIMIT;pf=pf+0.05)
           {
              for(n = 2; n <= N_LIMIT; n++)
                 {
                   Tn = get_t(n); *E(Tn)*
                   Mn = get_m(n); *E(Tn^2)*
                   tn[n] = Tn;
                   mn[n] = Mn;
                 }
                 min = 2;
                 for(i = 2; i <= N_LIMIT; i++)
                     {
                    if(tn[i]/i <= min)
                       {
                         min = tn[i]/i;
                         k = i;
                         smin = mn[i];
                         V = smin/pow((double)k,2.0)
                             -pow(min,2.0); *Var(Tn/n)*
                       }
                     }
                 fprintf(fp, "p:%.3f pf:%.3f n*:%d
                          E(Tn*/n*):%.10f
                          1/(E(Tn*/n*)):%.5f
                          var:%.5f cv:%.5f\n",p,pf,
                          k,min,1/min,V,
                          sqrt(V)/(Tn/n));
           }
     fclose(fp);
     return(0);
}
double  get_t(int n)
{
```

```
double  res1;
r=ceil(log((1-ALFA)*(1-pow(1-p,(double)n))/
  (ALFA*pow(1-p,(double)n)))/log(pf));;
r1=ceil(log(p*(1-ALFA)/((1-p)*ALFA))/log(pf));
A=1-(r1+1)*pow(pf,r1)+r1*pow(pf,(double)(r1+1));
C=p+(1-p)*pow(pf,(double)r1);
D=1+pf;
F=1-pf;
An=1-(r+1)*pow(pf,(double)r)+r*pow(pf,(double)(r+1));
Bn=pow(pf,(double)r);
P=r1*(1-pow(1-C,(double)n))+A*(1-p)*(1+pow(1-C,
  (double)(n-1))*(C*(1-n)-1))/(F*C)+A*(1-p)*
  n*pow(1-C,(double)(n-1))/F;
res1 =sum1(n)+(r+P)*(1-(1-Bn)*pow(1-p,(double)n))+
      An*pow(1-p,(double)n)/F;
return(res1);
}
double get_m(int n)
{
double res2;
r=ceil(log((1-ALFA)*(1-pow(1-p,(double)n))/
  (ALFA*pow(1-p,(double)n)))/log(pf));
r1=ceil(log(p*(1-ALFA)/((1-p)*ALFA))/log(pf));
A=1-(r1+1)*pow(pf,(double)r1)+r1*
  pow(pf,(double)(r1+1));
C=p+(1-p)*pow(pf,(double)r1);
D=1+pf;
F=1-pf;
B=pow(pf,(double)r1);
H=(D*A-r1*(r1+1)*B*pow(F,2.0))*(1-p)/pow(F,2.0);
An=1-(r+1)*pow(pf,(double)r)+r*pow(pf,(double)(r+1));
Bn=pow(pf,(double)r);
Hn=(D*An-r*(r+1)*Bn*pow(F,2.0))*pow(1-p,(double)n)/
   pow(F,2.0);
P=r1*(1-pow(1-C,(double)n))+A*(1-p)*(1+pow(1-C,
  (double)(n-1))*(C*(1-n)-1))/(F*C)+A*(1-p)*
   n*pow(1-C,(double)(n-1))/F;
S1=H/(1-C)+2*r1*A*(1-p)/(F*(1-C));
S2=1-(n+1)*pow(1-C,(double)n)+n*pow(1-C,(double)(n+1));
S3=pow(A*(1-p)/(F*(1-C)),2.0);
S=C*r1*(1-r1)+H*n*pow(1-C,(double)(n-1))+(n-1)*
  n*S3*pow(1-C,(double)n)+(1-pow(1-C,(double)n))*
  (2*S3-S1+pow(r1,2.0))+S3*(S2*(2-C)-n*(n+1)*
  pow(1-C,(double)n)*pow(C,2.0))/pow(C,2.0)+
  S2*(S1-3*S3)/C;
res2=Hn+(1-pow(1-p,(double)n)*(1-Bn))*(pow(r,2.0)+2*
     r*P+S)+sum2(n);
return (res2);
}
```

```
double  sum1(int n)
{
double  t;
int     j;
     t = 0;
     tn[1] =r1*C+(1-p)*A/F;
     for(j = 1; j <= n-1; j++)
t=t + (1-(1-Bn)*pow(1-p,(double)n))*C*
  pow(1-C,(double)(j-1))*tn[n-j];
return(t);
}
double sum2(int n)
{
double x;
int    y;
       x=0;
       tn[1] =r1*C+(1-p)*A/F;
       mn[1] =pow(r1,2.0)*C+H;
       for(y=1;y<=n-1;y++)
x=x+2*(1-pow(1-p,(double)n)*(1-Bn))*
  (r+r1+(y-1)*A*(1-p)/
  (F*(1-C)))*C*pow(1-C,(double)(y-1))*tn[n-y]+
  (1-pow(1-p,(double)n)*(1-Bn))*C*pow(1-C,(double)
  (y-1))*mn[n-y];
return (x);
}
```

Program 3 Calculates expected number of tests per sampl and corresponding variance for MD.

```
#include        <stdio.h>
#include        <math.h>
#define         ALFA    .98
#define         N_LIMIT  150

double  p,pf;
double  r,r1;
double  A,B,C,D,F,H,An,Bn,Hn,K,V;

main()
{
int     n;
double  T;
FILE    *fp;
     fp = fopen("Drive:Filename", "w");
     p=Prevalence Rate;
     pf=Probability of a False Positive Outcome;
       for(n=2; n<= N_LIMIT; n=n+1)
          {
```

```
            r1=ceil(log(p*(1-ALFA)/
               ((1-p)*ALFA))/log(pf));
            r=ceil(log((1-ALFA)*
              (1-pow(1-p,(double)n))/(ALFA*
               pow(1-p,(double)n)))/log(pf));
            A=1-pow(pf,(double)r1)*(1+r1)+r1*
              pow(pf,(double)(r1+1));
            An=1-pow(pf,(double)r)*(1+r)+r*
              pow(pf,(double)(r+1));
            B=pow(pf,(double)r1);
            Bn=pow(pf,(double)r);
            C=p+(1-p)*pow(pf,(double)r1);
            D=pf+1;
            F=1-pf;
            H=(D*A-r1*(r1+1)*B*pow(F,2.0))
              *(1-p)/pow(F,2.0);
            Hn=(D*An-r*(1+r)*Bn*pow(F,2.0))*
               pow(1-p,(double)n)/
               pow(F,2.0);
            K=pow(n,2.0)*(pow(r1,2.0)*C+H);
            T =(1-(1-Bn)*pow(1-p,(double)n))*   *E(Tn)*
              (r+n*(A*(1-p)/F+r1*C))+
              An*pow(1-p,(double)n)/F;
            V=(Hn+(1-(1-Bn)*pow(1-p,(double)n))*
              (pow(r,2.0)+K+2*r*n*              *Var(Tn/n)*
              (r1*C+(1-p)*A/F)))/pow(n,2.0)
               -pow(T/n,2.0);
            fprintf(fp, " n:%d E(Tn/n):%.5f
                    1/(E(Tn/n)):%.5f var:%.5f
                    cv:%.5f\n",n,T/n,n/T,V,
                    sqrt(V)/(T/n));
         }
      fclose(fp);
      return(0);

}
```

Program 4 Calculates minimum expected number of tests per sample and corresponding variance and group size for MD. (In this program parameters are set to the following values:
p=0.001...0.02, pf_{+}=0.05...0.5, n=2...150.)

```
#include        <stdio.h>
#include        <math.h>
#define         ALFA    .98
#define         N_LIMIT  150
#define         P_LIMIT (double) .02
#define         PF_LIMIT (double) .5
```

```
double  p;
double  pf;
double  r,r1;
double  A,B,C,D,F,H,An,Bn,Hn,K,V;
double  tn[N_LIMIT],mn[N_LIMIT];

main()
{
int     i,n,k;
double  T,min,smin;
FILE    *fp;
     fp = fopen("Drive:Filename", "w");
     for(p = .001; p <= P_LIMIT; p = p + .001)
          for(pf = .05; pf <= PF_LIMIT; pf = pf + .05)
               {
                for(n=2; n<= N_LIMIT; n=n+1)
                 {
                  r1=ceil(log(p*(1-ALFA)/((1-p)*
                     ALFA))/log(pf));
                  r=ceil(log((1-ALFA)*(1-pow(1-p,
                    (double)n))/(ALFA*pow(1-p,
                    (double)n)))/log(pf));
                  A=1-pow(pf,(double)r1)*(1+r1)
                    +r1*pow(pf,(double)(r1+1));
                  B=pow(pf,(double)r1);
                  Bn=pow(pf,(double)r);
                  An=1-pow(pf,(double)r)*
                    (r+1)+r*pow(pf,(double)(r+1));
                  C=p+(1-p)*pow(pf,(double)r1);
                  D=1+pf;
                  F=1-pf;
                  H=(D*A-r1*(r1+1)*B*pow(F,2.0))
                    *(1-p)/pow(F,2.0);
                  Hn=(D*An-r*(r+1)*Bn*pow(F,2.0))*
                     pow(1-p,(double)n)/pow(F,2.0);
                  K=pow(n,2.0)*(pow(r1,2.0)*C+H);
  *E(Tn)*         T =(1-(1-Bn)*pow(1-p,(double)n))*
                    (r+n*(A*(1-p)/F+r1*C))+An*
                    pow(1-p,(double)n)/F;
 *Var(Tn/n)*      V=(Hn+(1-(1-Bn)*pow(1-p,(double)n))*
                    (pow(r,2.0)+K+2*r*n*(r1*C+(1-p)*
                    A/F)))/pow(n,2.0)-pow(T/n,2.0);
                  tn[n]=T;
                  mn[n]=V;
                 }
                min=100;
                for (i=2;i<=N_LIMIT;i=i+1)
                {
```

```
                    if(tn[i]/i<=min)
                       {
                      min=tn[i]/i;
                      k=i;
                      smin=mn[i];
                       }
                   }
                   fprintf(fp, "p:%.3f pf+:%.3f n*:%d
                           E(Tn*/n*):%.5f
                           1/(E(Tn*/n*)):%.5f cv:%.5f\n",
                           p, pf,k,min,1/min,
                           sqrt(smin)/min);
               }
fclose(fp);
return(0);
}
```

Program 5 Calculates expected number of tests per sample and corresponding variance for MI. (In this program parameters are set to the following values: p=0.001...0.02, pf_+=0.05...0.5.)

```
#include        <stdio.h>
#include        <math.h>
#define         ALFA    .98
#define         P_LIMIT (double) 0.02
#define         PF_LIMIT (double) 0.5

double  p;
double  pf;
double  r1;
double  A,B,C,D,F,H;

main()
{
double  T,V;
FILE    *fp;
     fp = fopen("Drive:Filename", "w");
     for(p = 0.001; p <= P_LIMIT; p = p + .001)
          for(pf = 0.05; pf <= PF_LIMIT; pf = pf + .05)
               {
                  r1=ceil(log(p*(1-ALFA)/
                    ((1-p)*ALFA))/log(pf));
                  A=1-pow(pf,(double)r1)*(1+r1)
                    +r1*pow(pf,(double)(r1+1));
                  C=p+(1-p)*pow(pf,(double)r1);
                  F=1-pf;
                  D=1+pf;
                  B=pow(pf,(double)r1);
```

```
                    H=(D*A-r1*(r1+1)*B*
                      pow(F,2.0))*(1-p)/pow(F,2.0);
*E(T1)*             T =(1-p)*A/F+r1*C;
*Var(T1)*           V=pow(r1,2.0)*C+H-pow(T,2.0);
                    fprintf(fp, "p:%.5f pf+:%.5f
                            E(Tn/n):%.5f
                            1/(E(Tn/n)):%.5f var:%.5f
                            cv:%.5f\n",p, pf,T,
                            1.0/T,V,sqrt(V)/T);
                }
     fclose(fp);
     return(0);
}
```

Program 6 Calculates the number of retests.

```
#include        <stdio.h>
#include        <math.h>
#define         ALFA    .98
#define         N_LIMIT  300

double  p;
double  pf;

main()
{
int     n,r;
FILE    *fp;
     fp = fopen("Drive:Filename", "w");
     p = Prevalence Rate;
     pf = Probability of a False Positive Outcome;
     for(n=1; n<= N_LIMIT; n=n+1)
        {
          r=ceil(log((1-ALFA)*(1-
            pow(1-p,(double)n))/(ALFA*
            pow(1-p,(double)n)))/log(pf));
          fprintf(fp, "%d %d\n",n,r);
        }
fclose(fp);
return(0);
}
```

Program 7 Calculates minimum expected number of tests per sample and corresponding group size for the original Sterrett's procedure using the model described in this thesis.

```
#include        <stdio.h>
#include        <math.h>
#define         N_LIMIT 30

double  p;

double  sum1(int n);
double  get_t(int n);
double  tn[N_LIMIT];

main()
{
int     n;
double  Tn;
int     i ;
int     k;
double  min;
FILE    *fp;
     fp = fopen("Drive:Filename", "w");
     p=Prevalence Rate;
       for(n = 2; n <= N_LIMIT; n++)
              {
               Tn = get_t(n);              *E(Tn)*
               tn[n] = Tn;
              }

              min = 2;
              k = 2;
              for(i = 2; i <= N_LIMIT; i++)
                {
                   if(tn[i]/i <= min)
                      {
                       min = tn[i]/i;
                       k = i;
                      }
                }
              fprintf(fp, " n*:%d
                      E(Tn*/n*):%.10f\n",k, min);

     fclose(fp);
     return(0);

}
double  get_t(int n)
{
double  res1;
     res1 =sum1(n)+(p-pow(p,2.0)*pow(1-p,
          (double)(n-1))+1-(n+1)*
```

```
            pow(1-p,(double)(n))+n*
            pow(1-p,(double)(n+1)))/p;
return(res1);
}

double  sum1(int n)
{
double  t;
int     j;
     t = 0;
     tn[1] =1;
     for(j = 1; j <= n-1; j++)
     t = t + p*pow(1-p,(double)(j-1))*tn[n-j];
     return(t);
}
```

Program 8 Calculates minimum expected number of tests per sample and corresponding group size for original Sterrett's procedure using Sterrett's formula.

```
#include        <stdio.h>
#include        <math.h>
#define         K_LIMIT 20

double p;
double get_t(int);
double get_B(int);
double tk[K_LIMIT];

main()
{
 int k,i,m;
 double min,Tk;
 FILE       *fp;
    fp=fopen("Drive:Filename","w");
    p=Prevalence Rate;
     for(k=2; k <= K_LIMIT; k++)
      {
       tk[k]=get_t(k);    *E(Tn)*
      }
      min=2;
      m=2;
      for(i=2; i <= K_LIMIT; i++)
         {
           if(tk[i]/i <= min)
           {
            min=tk[i]/i;
            m=i;
```

```
            }
          }
      fprintf(fp,"k*:%d E(k*,p)/k*:%.10f \n",m,min);
 fclose(fp);
 return(0);
}

double get_t(int k)
{
  double t,A,B,k1;
  int j;
  k1=(double)k;
  t=pow(1-p,k1)+k1*p*pow(1-p,k1-1)*(2+(k1+1)/2-2/k1);
  for(j=2;j<=k;j++)
   {
     A=get_B(k);
     B=get_B(j)*get_B(k-j);
     t=t+pow(p,(double)j)*pow(1-p,k1-j)
     *(j*(k1+1)/(j+1)+j+1-2*j/k1)*A/B;
   }
  return(t);
}

double get_B(int j)
{
  int v;
  double l;

  l=1.0;
  for(v=1;v<j;v++)
     {
      l=l*(1+v);
     }
return(l);
}
```