1 New approach and new program for analyses of false negatives- 2 contaminated data in medicine and biology

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16 Abstract

17 **Background:** No serological assay has 100% sensitivity. Statistically, the concentration of specific antibodies
18 against antigens of parasites decreases with the duration of infection. This can result in false negative outputs of
19 diagnostic tests for the subjects with old infection, e.g., for individuals infected in childhood. When a property
20 of seronegative and seropositive subjects is compared under these circumstances, the statistical tests can detect
21 no significant difference between these two groups of subjects, despite the fact that infected and noninfected
22 subjects differ. When the effect of the infection has a cumulative character and subjects with an older infection
23 (potential false negatives) are affected to a greater degree, we can even get paradoxical result of the comparison
24 – the seronegative subjects have on average lower value of certain traits, e.g. IQ, despite the infection having a
25 negative effect on the trait. A permutation test for the contaminated data, implemented, e.g., in the program
26 Treetpt or available as a comprehensibly commented R function in the supplement of this paper, can be used to
27 reveal and to eliminate the effect of false negatives.

28 **Methods:** We used a Monte Carlo simulation in the program R to show that the permutation test implemented in
29 the programs Treetpt and PTPT is a conservative test.

30 **Results:** We showed that the test could provide false negative but not false positive results if the studied
31 population contains no subpopulation of false negative subjects. We also introduced R version of the test
32 expanded by skewness analysis, which helps to estimate the proportion of false negative subjects based on the
33 assumption of equal data skewness in groups of healthy and infected individuals.

34 **Conclusions:** Based on the results of simulations and our experience with empirical studies we recommend the
35 usage of permutation test for contaminated data whenever seronegative and seropositive individuals are
36 compared.

37 **Keywords:** randomisation tests; epidemiology; serology; case-control studies; specificity; sensitivity;
38 toxoplasma

39

40 Introduction

41 The reported decrease of specific antibodies with time from the onset of infection increases the risk of
42 false negative test results in subjects with old infections, e.g., in individuals infected in childhood¹⁻³. This is also
43 true for parasites that stay dormant in infected cells until the end of the life of infected hosts. Any subsample of
44 seronegative subjects could therefore be contaminated with an unknown proportion of misdiagnosed parasite-
45 positive individuals who got infected a long time ago⁴⁻⁶. This subpopulation of infected but seronegative subjects
46 could be the most influenced by the infection (Figure 1B) because of the long duration of their infection or
47 because their infection took place in early stages of their ontogenesis. This could result in a paradox (Figure 1C).
48 The seropositive subjects could have on average higher IQ scores (or higher body weight) while the intelligence
49 (or body weight) of seropositive subjects declines with the assessed length of infection (obtained from clinical
50 records or assessed by the level of antibodies).

51 **Figure 1. Exemplar distributions under 3 different contamination levels.**

52

53 *The proportion of seropositive individuals (50%), the difference between healthy and infected individuals (5) and*
 54 *the standard deviation (10, corresponding to Cohen's $d = 0.5$ in non-contaminated sample) within healthy*
 55 *individuals are held constant. Histogram C serves as a demonstration of the paradoxical result caused by a high*
 56 *contamination when the seronegative is a lower seropositive mean trait value despite the fact that healthy*
 57 *individuals score higher than infected individuals.*

58 The contamination of a parasite-free subsample with false negative individuals can be revealed and
 59 eliminated by permutation tests with the reassignment of suspect cases between subsamples^{4,5}. Such permutation
 60 tests can be performed using the program Treept, originally called PTPT^{7,8} modified for an analysis of data
 61 contaminated with an unknown number of subjects with false negative diagnosis using the method of

reassignment of potentially false negative subjects⁴. This freeware program is available at <http://web.natur.cuni.cz/flegr/treept.php>. The updated version of the test suited for R can be found in the supplementary material of this paper in the form of comprehensibly commented R script.

The algorithm of the one-tailed permutation test with data reassignment is as follows: Particular percentage (e.g. 5, 10, 15, 20 or 25 %) of subjects with the lowest (highest) value of the dependent variable, for example IQ score, is relocated from the group of parasite-seronegative subjects to the group of the parasite-seropositive subjects. Then, the difference of means of these two groups is calculated. In the next 9,999 steps, the empirical values of the analysed variable are arbitrarily assigned into two groups held at the size of the original seronegative and seropositive groups. The particular percentage of cases with the lowest (or highest) values of the focal variable (e.g. IQ) in the pseudoseronegative group is relocated to the pseudoseropositive group, and the difference between the means of the two groups is calculated. Finally, all 10,000 differences (including the one calculated from non-permuted data) are sorted from highest to lowest. The percentage of the differences higher or equal to that calculated on the basis of the non-permuted data is considered as the statistical significance (p) – the probability of obtaining the same or higher difference between the means of two groups, if the null hypothesis is correct and subjects are assigned into seropositive and seronegative groups randomly.

Our main aim is to show that the permutation test for contaminated data does not provide false positive results, i.e., it does not return lower p than a standard permutation test if no false negative subjects exist in the studied population. The second aim is to develop a new tool for the skewness analysis, which can be used to estimate the approximate proportion of false-negative subjects in the studied population.

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Methods and Results

A Monte Carlo simulation was performed with R 3.3.3. We generated a population of 150 parasite-free and 150 infected subjects (mean intelligence was 101.5 in the parasite-free group and 98.5 in the infected group – the between-group difference was 3, the population mean intelligence was 100). Subjects were normally distributed around group means with equal standard deviations (SD). We used different SDs (6, 9, 12, 15, 30) corresponding to different effect sizes expressed by Cohen's d (0.5, 0.33, 0.25, 0.2, 0.1). Then we ran a standard permutation test. We randomly permuted the infection status of all subjects 10,000 times and calculated a fraction of permutations where the difference between two groups (pseudo-parasite-free and pseudo-parasite-infected subjects) was equal to or larger than the difference between the groups in non-permuted data (p value of a standard permutation test). Then, we repeated the analysis using a one-tailed permutation test for contaminated data. Namely, after the generation of sets of parasite-free and parasite-infected subjects (or after the generation of sets of pseudo-parasite-free and pseudo-parasite-infected subjects by permutation of the infection status), we relocated 5, 10, 15, 20, 25, 30 or 50% of subjects with the lowest intelligence from the parasite-free (or pseudo-parasite-free) set to the parasite-infected (or pseudo-parasite-infected) set. Again, we calculated a fraction of permutations with the difference between the groups equal to or larger than the value computed for the non-permuted data (p values of the permutation test for contaminated data). We used populations generated for the standard permutation test (each initial population was used once for each fraction of relocated subjects). In total, 10,000 original populations were generated for each SD, therefore 10,000 independent permutation tests were

conducted for each combination of SD and each relocated fraction. The resulting p values were averaged over permutation tests with the same population SD and the same relocated fraction. The results are shown in the Table 1. With the proportion of relocated subjects, the average p-value grew for every standard deviation. The visualization of this growth can be found in Figure 2A. In this figure, the p-value of the standard permutation test was subtracted from each p-value of the permutation test for contaminated data (negative values therefore correspond to a decrease, and positive to an increase, of p-value in comparison to a standard permutation test).

When several exceptional data points (outliers) are present, the p-value of one or more contamination levels can be lower than p-value for 0% contamination. This is more frequent when the effect size is very small and the p-value fluctuates due to a larger impact of random noise in the data. The probability of a p-value being higher for a certain proportion of relocated data than the p-value of a standard permutation test in a particular simulation run was evaluated for each level of contamination and SD from the set of generated data described above. The results are reported in the Table 3 and shown in the Figure 3A.

For comparison, the same computer simulation was conducted for a population of 150 seropositive and 150 seronegative individuals where 5% of seronegative individuals were false negative individuals with extremely low intelligence (example in Figure 1B). The average p-values of the permutation test for contaminated data are in Table 2. The graphical representation of the difference between a p-value for 0 % of relocated subjects and other contamination levels is represented in Figure 2B, and the probability of increase of p-value is shown in Table 4 and Figure 3B. In this case, the p-value decreases with the proportion of relocated individuals as expected.

Table 1 Effect of relocation of hypothesized false negative subjects on the results of a permutation test if no such subjects exist in the population

SD	Fraction of relocated subjects							
	0 %	5 %	10 %	15 %	20 %	25 %	30 %	50 %
6	.001	.001	.001	.002	.002	.002	.002	.005
9	.021	.022	.023	.024	.026	.028	.030	.044
12	.064	.066	.068	.070	.073	.076	.080	.099
15	.108	.110	.113	.116	.119	.123	.126	.148
30	.269	.270	.272	.274	.277	.280	.283	.298

The table shows p-values computed with the permutation test for contaminated data when the population under study contains no false negative subjects. The simulation experiments were performed on populations that differ by variances (rows) with the relocation of different fractions of IQ-lowest individuals (columns) from the high-IQ (seronegative) group to the low-IQ (seropositive) group. The first column (0%) shows the (most significant) results of permutation tests performed without any relocation of data. For details see the Methods section. The fixed effect was 3 IQ points. The population size was 300, and the proportion of seropositive individuals in the original sample (0% relocation) was 0.5.

Table 2 Effect of the relocation of hypothesized false negative subjects on the results of a permutation test if 5% of such individuals is present in seronegative group

SD	Fraction of relocated subjects							
	0 %	5 %	10 %	15 %	20 %	25 %	30 %	50 %
6	.058	.035	.022	.017	.015	.014	.014	.017
9	.273	.208	.157	.131	.116	.107	.102	.100
12	.446	.371	.303	.263	.236	.219	.209	.191
15	.565	.491	.418	.371	.337	.315	.302	.274
30	.775	.721	.659	.610	.571	.542	.523	.467

The table shows p-values computed with the permutation test for contaminated data when the seronegative group contains 5% of false negative subjects. The simulation experiments were performed on populations that differ by variances (rows) with relocation of different fractions of IQ-lowest individuals (columns) from the high-IQ (seronegative) group to the low-IQ (seropositive) group. The first column (0%) shows the (least significant) results of permutation tests performed without any relocation of data. For details see the Methods section. The fixed effect was 3 IQ points. The population size was 300, and the proportion of seropositive individuals in the original sample (0% relocation) was 0.5.

Table 3. The probability of a p-value being higher in a particular simulation run than a p-value with 0 % of relocated individuals. No false negative subjects are present in the population.

SD	Fraction of relocated subjects						
	5 %	10 %	15 %	20 %	25 %	30 %	50 %
6	.18	.23	.27	.31	.35	.39	.58
9	.45	.50	.54	.58	.61	.64	.75
12	.50	.54	.57	.60	.62	.64	.71
15	.51	.55	.57	.59	.61	.63	.68
30	.50	.52	.53	.54	.55	.56	.59

The probability that p-value will increase for specified fraction of relocated individuals in a particular simulation run as compared to 0 % of relocated seronegative individuals. The simulated population are identical to the population represented in Table 1. The graphical summary can be found in Figure 3A. The relatively small numbers in first row are caused by the fact that in many simulation runs p-values remained <.0001 for a small fraction of relocated subjects when the effect size is relatively large.

Table 4. The probability of p-value being higher in particular simulation run than p-value with 0 % of relocated individuals. 5% of seronegative group are false negative individuals.

SD	Fraction of relocated subjects						
	5 %	10 %	15 %	20 %	25 %	30 %	50 %
6	.00	.00	.00	.00	.00	.00	.06
9	.00	.00	.00	.00	.00	.00	.03
12	.00	.00	.00	.00	.00	.00	.02
15	.00	.00	.00	.00	.00	.00	.02
30	.00	.00	.00	.00	.00	.00	.01

The probability that a p-value will increase for a specified fraction of relocated individuals in a particular simulation run as compared to 0 % of relocated seronegative individuals. The simulated population are identical to those represented in Table 2. The graphical summary can be found in Figure 3B.

Two equivalent simulations were run to demonstrate the permutation test for contaminated data on the paradoxical dataset with a high proportion of false negative individuals. The first population of 150 seropositive and 150 seronegative individuals where 20% of seronegative subjects were false negative individuals with extremely low intelligence (Figure 1C). A similar one-tailed permutation test as in previous simulations was run as it was hypothesised that the average trait value of the healthy group is actually higher despite the paradoxical situation. The graphical representations of the results are in Figure 2C and Figure 3C. The second test with the same sample generation algorithm (150 seropositive, 150 seronegative, 20% false negative) was set to follow the default setting of the permutation test for contaminated data, which assumes the non-paradoxical situation and therefore relocates seronegative individuals with high trait value, thus widening the gap between the groups. Yielded p-value of one-tailed permutation test is then the proportion of random samples after relocation where the difference between groups (seronegative - seropositive) was lower than in the original sample (Figure 2D and Figure 3D). In both simulation tests on a sample with 20% contamination, the p-value of respective one-tailed permutation test decreased, so this sample was clearly distinguishable from the case in which no false negative subjects were present.

The appropriate direction of subject relocation can be determined on the basis of a skewness analysis of the original sample, which is available in the R version of the test⁹ if a parameter skewness.analysis is set to TRUE. The skewness analysis and its usage for the assessment of group mean order as well as the contamination level estimation is described in the Appendix of this paper. Using a two-tailed test is also worth consideration in this case. The p-value is then declining with the proportion of relocated individuals in all cases where false negative individuals are present (in well identified paradoxical situations only after the group means change their order into the right direction).

Figure 2: Heatmap of the average difference between the p-value of standard permutation test and p-value of the respective permutation test for contaminated data.

One-tailed tests were used. The p-value increases with the fraction of relocated individuals if no actual false negative individuals are present (A) and decreases if the sample is contaminated (B, C). This is true even if the wrong relocation direction is employed due to a paradoxical switch in the order of group means (D).

Figure 3: Heatmap of probability of p-value increase in particular simulation run.

The p-value does not increase in 100% of non-contaminated samples when a permutation test for contaminated data is used, but the probability that it happens is very high compared to samples in which false negative individuals occur.

Conclusions

The results of simulation showed that the permutation test for contaminated data does not provide more significant results than a standard permutation test if the experimental data does not contain a subpopulation of false negative subjects. This test is conservative when its usage is not necessary and allows one to avoid false negative results in the case of data contamination. This is due to the higher difference between the relocated seronegative and the original seropositive group in the presence of false negative data. The referential set of permutations with relocation remains the same in both cases, while the relative change in inter-group difference

after relocation maintains an intermediate position between those two options (see Figure 4). Therefore, the positive result of this test, i.e. lowering the p-value with the growth of the proportion of relocated individuals, itself supports the hypothesis that the set of seemingly parasite-free subjects contains false negative subjects, who, most probably, have become infected a long time ago or in very young age.

Figure 4. Graphical demonstration of the intermediate position of referential permutations with relocation between empirical cases of relocation of seronegative healthy subjects and false negative infected subjects.

The increase in p-value in the case of non-contaminated data is much smaller than the increase caused by possible contamination, which can completely wipe out the actual inter-group difference or even cause a paradoxical switch of the group mean order. (See Table 5 or the position of 0 in the legend of Figure 2.)

Table 5. Risk associated with different combinations of data and used permutation tests

		Test	
		regular permutation test	permutation test for contaminated data
Data	non-contaminated	No risk	Small risk of false negative results
	contaminated	High risk of false negative or paradoxical results	No risk

The pressure to avoid a high risk associated with regular permutation test will lead us to the universal utilization of permutation test for contaminated data whenever properties of seropositive and seronegative subjects are compared. When we conduct a skewness analysis for contaminated data (see the Appendix) prior to the permutation test, we can lower the risks further by justification of regular permutation test or informed setting of relocated fractions of seronegative individuals in permutation test for contaminated data.

Based on the theoretical grounding described above and our experience with the research of two unrelated species of parasites, *Toxoplasma gondii*^{5, 10} and human cytomegalovirus⁶, we strongly recommend the usage of permutation tests for contaminated data⁹ whenever any properties of parasite-infected and parasite-free individuals are compared.

Conflict of interest statement

The authors declare to have no conflicts of interest.

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Appendix 1

Estimation of the fraction of false negatives by skewness analysis

The estimation of the actual contamination level is very difficult to discern and should be investigated more in future research. For now, we can seek assistance in a skewness analysis, which compares the skewness of trait value distribution in seropositive and seronegative groups. Skewness is defined as third standardized moment measuring the asymmetry of the probability distribution. We assume that healthy and infected individuals have an equally skewed trait distribution. This assumption is violated if false negative individuals are recruited from one of extreme tails of the distribution of infected individuals. If, for example, infected individuals with the lowest trait value are identified as seronegative (as seen in Figure 1B), the skewness of seropositive individuals becomes more positive and the skewness of seronegative individuals more negative. The exact opposite is true if individuals from the upper tail of the distribution are misdiagnosed as negative. The skewness comparison (available as a function in supplementary R script) of contaminated data compares Fisher-Pearson coefficient of skewness of seropositive and seronegative groups under different hypothesised contamination levels and returns the skewness values for each fraction of relocated subjects, p-values of the difference between them based on permutation test, the interval where the group skewness is not significantly different and a proportion of relocated seronegative individuals at which the difference between group skewness was smallest (i.e. the one the generated the most similarly skewed groups). This value generally underestimates the actual contamination, but any amendments would require additional assumptions about the distribution of healthy/infected individuals, which would not be necessarily met in empirical data. **Now we recommend the conduction a skewness comparison prior to the evaluation of the between-group difference and then the conduction of a permutation test for contaminated data for contamination levels between 0 and upper border of the interval, where the difference between group skewness was not significant.** We observed that the actual level of contamination in simulated data, where we can control the contamination level, rather closely matches the upper level of the similar-skewness interval due to the fact that the distributions of healthy and infected subjects largely overlap, and the extreme tail of seronegative distribution contains also extreme healthy individuals which are relocated prior to actual false negative individuals. For the same reason, however, we can suggest that the between-group difference for the relocated fraction where the group skewness are most similar (described above) closely matches the actual between-group difference in non-contaminated populations without false negative subjects.

The difference between skewness coefficients in seropositive and seronegative groups in the original sample without relocated individuals can also be evaluated in the R version of the permutation test for contaminated data⁹ (set skewness.analysis to TRUE). This analysis allows one to appropriately assess whether the seronegative group includes false negative subjects from the extreme tail of the distribution of infected individuals. By default, the permutation test for contaminated data assumes that the observed order of mean values of seropositive and seronegative groups accurately reflects the state of things in correctly determined healthy and infected groups. Therefore, the function will gradually relocate individuals from the lower tail of the distribution if the seronegative mean trait value is higher than the seropositive mean and vice versa (this can be changed by the parameter higher.healthy). If we do not alter default setting in paradoxical situations (Figure 1C), in which the order of group means was changed due to contamination, the test algorithm will increase the

difference between the groups by relocating healthy individuals from the upper tail of distribution of seronegative subjects. The p-value will most likely decrease with the growing fraction of relocated individuals, as in other cases where false negative individuals are present. This might lead to a radical misinterpretation of the data (confirmation of the assumption of higher trait value in group of infected individuals) if attention is not paid to the skewness analysis. The skewness analysis of the original sample is not fooled as easily since mismatching the extreme tail of infected individuals as seronegative will alter the skewness of both groups substantially. Under an extreme proportion of false negatives, the skewness of both groups might actually be shifted in the same direction (e.g. positive in cases similar to the example in Figure 1C). However, the skewness of seropositive group will be still substantially more deflected than the skewness of the seronegative group, so the skewness analysis will return reliable results.

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Supplements (R scripts)

(Jaroslav Flegr and Petr Tureček, New approach and new program for analyses of false negatives-contaminated data in medicine and biology)

Supplement 1

Permutation test for contaminated data

```
#####

####hit ctrl+a and ctrl+r to install the function####

#####

#This script contains contamination_perm_test() function

#contamination_perm_test(trait,identification,percentages=c(0,5,10),higher.
healthy=(mean(trait[identification==F])>mean(trait[identification==T])),run
s=10000,skewness.analysis=F)

#Arguments are described below.

#It is necessary to define funtion calculating skewness index first:

##Fuction that returns Fisher-Pearson coefficient of skewness.

##Input is a vector of numerical values.

FPskewness<-function(x){
  return((sum((x-mean(x))^3)/length(x))/((sqrt(sum((x-
mean(x))^2)/length(x)))^3))
}

###contamination_perm_test

###Function that delegates the parameters to either one-tailed or two-
tailed tests described below
```

```

341 contamination_perm_test<-
342 function(trait,identification,percentages=c(0,5,10),higher.healthy=(mean(tr
343 ait[identification==F])>mean(trait[identification==T])),runs=10000,two.tail
344 ed=F,skewness.analysis=F){
345   if(two.tailed==F){
346     contamination_perm_test_one(trait=trait,identification=identification,perce
347 ntages=percentages,higher.healthy=higher.healthy,runs=runs,skewness.analysi
348 s=skewness.analysis)
349   }else{
350     contamination_perm_test_two(trait=trait,identification=identification,perce
351 ntages=percentages,higher.healthy=higher.healthy,runs=runs,skewness.analysi
352 s=skewness.analysis)
353   }
354 }
355
356 ###This Function works with following arguments:
357 ###trait - Numerical vector of trait values
358 ###identification - Logical vector of assumed presence (T) or absence (F)
359 of infection
360 ###percentages - Numerical vector of percentages of false negative amongst
361 negative subjects (contamination levels) for which the permutation test for
362 contaminated data will be run.
363 ###two.tailed - Specifies the verison of the test, two.tailed=F is the
364 default.
365 ###higher healthy - Logical. Indicates whether we assume the healthy
366 individuals to show higher (T) or lower (F) trait values. When not
367 specified, the script assumes this relationship based of group means with
368 no hypothesised contamination.
369 #####It allows us to use the difference between the groups (not in
370 absolute values) in permutation test. In this scenario the seropositive
371 group mean is substracted from seronegative group mean and the one-tailed
372 permutation test is conducted accordingly.
373 ###runs - Number of resamplings used in permutation test
374
375
376 ###One-tailed version of the test
377

```

```

378 contamination_perm_test_one<-
379 function(trait,identification,percentages=c(0,5,10),higher.healthy=(mean(tr
380 ait[identification==F])>mean(trait[identification==T])),runs=10000,skewness
381 .analysis=F){
382
383 if(length(trait)!=length(identification)){
384 stop("The vectors of trait values and infection indication are of different
385 lengths.")
386 }
387
388 higher<-(mean(trait[identification==F])>mean(trait[identification==T]))
389 set.higher<-higher.healthy
390
391 orig.means<-tapply(trait,identification,mean)
392 orig.means<-data.frame(orig.means)
393
394 names(orig.means)<-"Original mean values"
395 rownames(orig.means)[which(rownames(orig.means)=="FALSE")]<-"Identified as
396 healthy"
397 rownames(orig.means)[which(rownames(orig.means)=="TRUE")]<-"Identified as
398 infected"
399
400 higher.report<-ifelse(higher==T,
401 "In original sample, individuals identified as healthy showed higher
402 \naverage trait value.",
403 "In original sample, individuals identified as infected showed higher
404 \naverage trait value."
405 )
406
407 concord<-ifelse(higher==set.higher,"Consequently,","Despite that,")
408
409 set.report1<-paste(concord,ifelse(set.higher==T,
410 "healthy individuals were hypothesised to have higher \naverage trait value
411 in a contamination-free sample. \n",

```



```

412 "infected individuals were hypothesised to have higher \naverage trait
413 value in a contamination-free sample. \n"
414 ))
415
416 set.report2<-paste(ifelse(set.higher==T,
417 "\nFor each contamination level respective proportion of seronegative
418 \nindividuals with lowest trait value was relabeled as seropositive \nin
419 original sample as well as in each permutation test run.",
420 "\nFor each contamination level respective proportion of seronegative
421 \nindividuals with highest trait value was relabeled as seropositive \nin
422 original sample as well as in each permutation test run."
423 ))
424
425 trait<-c(trait[identification==F],trait[identification==T])
426 infected<-sort(identification)
427
428 count.healthy<-sum(!identification)
429 count.infected<-sum(identification)
430
431 Nperc<-length(percentages)
432
433 vector.ident<-list()
434
435 for(i in 1:Nperc){
436   reassign<-round(count.healthy*(percentages[i]/100))
437   identification<-c(rep(F,count.healthy-
438   reassign),rep(T,count.infected+reassign))
439   vector.ident[[i]]<-identification
440 }
441
442 which.test<-paste("One-tailed permutation test for contaminated data was
443 executed. \nProportion of differences (mean of non-infected - mean of
444 infected)",
445   ifelse(set.higher==T,"\nhigher","\nlower"),

```

```

446 "than the observed difference is returned as an equivalent \nof p-
447 value.\n",collapse=" ")
448
449 #Sorts healthy individuals to indicate possible false-negatives
450 trait2<-
451 c(sort(trait[infected==F],decreasing=higher.healthy),trait[infected==T])
452
453 dist.reals<-1:Nperc
454 names(dist.reals)<-paste(as.character(percentages), "%")
455
456 contamination<-paste(as.character(percentages), "%")
457 names(contamination)<-paste(as.character(percentages), "%")
458
459 mean.healthy<-dist.reals
460 mean.infected<-dist.reals
461
462 sd.healthy<-dist.reals
463 sd.infected<-dist.reals
464
465 N.healthy<-dist.reals
466 N.infected<-dist.reals
467
468 mean.dist.perm<-dist.reals
469 p.vals.perm<-dist.reals
470
471 #Compute group means in non-permuted sample
472 for(i in 1:Nperc){
473 mean.healthy[i]<-mean(trait2[vector.ident[[i]]==F])
474 mean.infected[i]<-mean(trait2[vector.ident[[i]]==T])
475 sd.healthy[i]<-sd(trait2[vector.ident[[i]]==F])
476 sd.infected[i]<-sd(trait2[vector.ident[[i]]==T])

```

```

477   N.healthy[i]<-sum(vector.ident[[i]]==F)
478   N.infected[i]<-sum(vector.ident[[i]]==T)
479   dist.reals[i]<-(mean(trait2[vector.ident[[i]]==F]) -
480   mean(trait2[vector.ident[[i]]==T]))
481 }
482
483 cohen<-
484 abs(dist.reals)/((sd.healthy*N.healthy+sd.infected*N.infected)/(N.healthy+N
485 .infected))
486
487 #Skewness computation
488 skewness.healthy<-FPskewness(trait[infected==F])
489 skewness.infected<-FPskewness(trait[infected==T])
490
491 skew.diff<-abs(skewness.healthy-skewness.infected)
492
493 skewness<-c(skewness.healthy,skewness.infected)
494 skewness<-data.frame(skewness)
495
496 names(skewness)<-"Fisher-Pearson coefficient of skewness"
497 rownames(skewness)<-c("Identified as healthy","Identified as infected")
498
499 signs<-sign(dist.reals)
500
501 #Permutation test with skewness add-on
502 perm.dist<-array(NA,dim=c(Nperc,runs))
503 rand.skew<-NA
504
505 for(run in 1:runs){
506   trait2<-sample(trait)
507   rand.skew[run]<-abs(FPskewness(trait2[infected==F]) -
508   FPskewness(trait2[infected==T]))

```

```

509
510 trait2<-
511 c(sort(trait2[infected==F],decreasing=higher.healthy),trait2[infected==T])
512
513 for(i in 1:Nperc){
514   perm.dist[i,run]<-mean(trait2[vector.ident[[i]]==F]) -
515   mean(trait2[vector.ident[[i]]==T])
516 }
517 }
518
519 skew.p<-sum(rand.skew>skew.diff)/runs
520
521 skew.higher<-ifelse(skewness.healthy>skewness.infected,"test-
522 negative","test-positive")
523 skew.guess.higher<-ifelse(skewness.healthy>skewness.infected,FALSE,TRUE)
524 healthy.positive<-ifelse(skewness.healthy>0,TRUE,FALSE)
525 infected.positive<-ifelse(skewness.infected>0,TRUE,FALSE)
526 skew.sig<-ifelse(skew.p<0.05,TRUE,FALSE)
527
528 skew.message<-paste(
529   ifelse(healthy.positive==infected.positive,
530     paste(
531       "The distribution of trait value was",
532       ifelse(healthy.positive,"positively","negatively"),
533       "skewed \nin both groups.",
534       "The Fisher-Pearson coefficient of skewness \nwas higher
535 in",skew.higher,"group.")
536 ,
537   paste("The distribution of individuals identified as healthy \nwas skewed",
538     ifelse(healthy.positive,"positively","negatively"),
539     "the distribution of individuals \nidentified as infected",
540     ifelse(infected.positive,"positively","negatively"))

```

```

541 )
542 ,
543 paste("\n\nThe difference between the coefficients of skewness was",
544 ifelse(skew.sig," \nstatistically significant.\n"," \nhowever, not
545 statistically significant.\n"),
546 "(Two-tailed permutation test of skewness difference \non ",runs," runs was
547 executed.)",sep="")
548 ,
549 ifelse(skew.sig==FALSE,
550 "\n\nThis might question the assumption of data contamination \nsince we
551 would expect a difference in skewness between \nthe groups in contaminated
552 data. \nProceed with caution.",
553 paste("\n\nThis supports the assumption of data contamination.",
554 "\nBased on the difference in skewness we would assume \ncontamination of
555 healthy group by false negative \nsubjects from the",
556 ifelse(skew.higher=="test-positive","lower","upper"),
557 "tail of the distribution \nof infected individuals, which would lead to
558 overall",
559 ifelse(skew.higher=="test-positive","\ndecrease","\nincrease"),
560 "of test-negative group mean."))
561 ,
562 ifelse(skew.sig==FALSE,"",
563 paste(ifelse(set.higher==skew.guess.higher,
564 paste("\n\nThe skewness analysis brings further support to the hypothesis
565 \nof",
566 ifelse(set.higher,"higher","lower"),
567 "mean in non-contaminated group of healthy \nindividuals, which was used in
568 current permutation test \nfor contaminated data.\n"),
569 paste("\n\nThe skewness analysis, however, does not support the hypothesis
570 \nof",
571 ifelse(set.higher,"higher","lower"),
572 "mean in non-contaminated group of healthy \nindividuals, which was used in
573 current permutation test \nfor contaminated data. Proceed with caution.\n")
574 )))
575 )

```

```

576
577   skewness<-rbind(skewness[1], "", skew.p)
578
579   rownames(skewness)[c(3,4)]<-c("", "p-value")
580
581   skewness[c(1,2,4),1]<-format(round(as.numeric(skewness[c(1,2,4),1]),3))
582
583   mean.dist.perm<-rowMeans(perm.dist)
584
585   if(higher.healthy==T){
586     for(i in 1:Nperc){
587       p.vals.perm[i]<-sum(dist.reals[i]<perm.dist[i,])/runs
588     }
589   }else{
590
591     for(i in 1:Nperc){
592       p.vals.perm[i]<-sum(dist.reals[i]>perm.dist[i,])/runs
593     }
594   }
595
596   mean.healthy<-format(round(mean.healthy,2))
597   mean.infected<-format(round(mean.infected,2))
598   sd.healthy<-format(round(sd.healthy,2))
599   sd.infected<-format(round(sd.infected,2))
600   cohen<-format(round(cohen,2))
601   dist.reals<-format(round(dist.reals,2))
602
603   mean.dist.perm<-format(round(mean.dist.perm,2))
604   p.vals.perm<-format(round(p.vals.perm,3))
605

```

```

606
607 res.table<-
608 rbind(contamination,mean.healthy,mean.infected,dist.reals,mean.dist.perm,sd
609 .healthy,sd.infected,N.healthy,N.infected,cohen,p.vals.perm)
610 res.table<-as.data.frame(res.table)
611
612 colnames(res.table)<-NULL
613 rownames(res.table)<-c("contamination","non-infeceted mean","infected
614 mean","mean difference","expected difference","non-infeceted SD","infected
615 SD","non-infeceted N","infected N","Cohen's d","p-value")
616
617 final.message<-ifelse(all(signs>0),
618 "\nThe mean difference was positive in all \nhypothesised contamination
619 levels.",
620 ifelse(all(signs<0),
621 "\nThe mean difference was negative in all \nhypothesised contamination
622 levels.",
623 ifelse(signs[1]>0,
624 "\nThe mean difference started as positive, but turned negative \nwith
625 growing hypothesised contamination level. \nThe results should be
626 interpreted with caution. \nRunning the test that assumess the opposite
627 relationship \nbetween group means (higher.healthy=T) or a two tailed test
628 \nis worth consideration.",
629 "\nThe mean difference started as negative, but turned positive \nwith
630 growing hypothesised contamination level. \nThe results should be
631 interpreted with caution. \nRunning the test that assumess the opposite
632 relationship \nbetween group means (higher.healthy=F) or a two tailed test
633 \nis worth consideration."
634 )))
635
636 cat("\nSample characteristics:\n")
637 print(orig.means)
638 cat(paste("\n",higher.report,"\n\n",sep=""))
639 cat(set.report1)
640
641 if(skewness.analysis==T){

```

```

642   cat("\n\nSkewness report:\n")
643   print(skewness)
644   cat("\n")
645   cat(skew.message)
646 }
647
648   cat("\n\nPermutation test for contaminated data:\n")
649   cat(paste(set.report2, "\n\n", sep=""))
650   cat(which.test)
651   cat(paste("\n", runs, "sample permutations were performed.\n"))
652   print(res.table)
653   cat(paste(final.message, "\n\n", sep=""))
654
655   results<-
656   list(orig.means, higher.report, set.report1, skewness, skew.message, set.report2
657   , which.test, res.table, final.message)
658
659   return(invisible(results))
660 }
661
662
663
664   ###Two-tailed version of the test:
665
666   contamination_perm_test_two<-
667   function(trait, identification, percentages=c(0,5,10), higher.healthy=(mean(tr
668   ait[identification==F])>mean(trait[identification==T])), runs=10000, skewness
669   .analysis=F){
670
671   if(length(trait)!=length(identification)){
672     stop("The vectors of trait values and infection indication are of different
673     lengths.")
674   }

```



```

675
676 higher<- (mean(trait[identification==F])>mean(trait[identification==T]))
677 set.higher<-higher.healthy
678
679 orig.means<-tapply(trait,identification,mean)
680 orig.means<-data.frame(orig.means)
681
682 names(orig.means)<-"Original mean values"
683 rownames(orig.means)[which(rownames(orig.means)=="FALSE")]<-"Identified as
684 healthy"
685 rownames(orig.means)[which(rownames(orig.means)=="TRUE")]<-"Identified as
686 infected"
687
688 higher.report<-ifelse(higher==T,
689 "In original sample, individuals identified as healthy showed higher
690 \naverage trait value.",
691 "In original sample, individuals identified as infected showed higher
692 \naverage trait value."
693 )
694
695 concord<-ifelse(higher==set.higher,"Consequently,","Despite that,")
696
697 set.report1<-paste(concord,ifelse(set.higher==T,
698 "healthy individuals were hypothesised to have higher \naverage trait value
699 in a contamination-free sample. \n",
700 "infected individuals were hypothesised to have higher \naverage trait
701 value in a contamination-free sample. \n"
702 ))
703
704 set.report2<-paste(ifelse(set.higher==T,
705 "\nFor each contamination level respective proportion of seronegative
706 \nindividuals with lowest trait value was relabeled as seropositive \nin
707 original sample as well as in each permutation test run.",

```

```

708  "\nFor each contamination level respective proportion of seronegative
709  \nindividuals with highest trait value was relabeled as seropositive \nin
710  original sample as well as in each permutation test run."
711  ))
712
713  trait<-c(trait[identification==F],trait[identification==T])
714  infected<-sort(identification)
715
716  count.healthy<-sum(!identification)
717  count.infected<-sum(identification)
718
719  Nperc<-length(percentages)
720
721  vector.ident<-list()
722
723  for(i in 1:Nperc){
724    reassign<-round(count.healthy*(percentages[i]/100))
725    identification<-c(rep(F,count.healthy-
726    reassign),rep(T,count.infected+reassign))
727    vector.ident[[i]]<-identification
728  }
729
730  which.test<-paste("Two-tailed permutation test for contaminated data was
731  executed. \nProportion of differences (in absolute value)",
732  "higher than the \nobserved difference is returned as an equivalent of p-
733  value.\n",collapse=" ")
734
735  #Sorts healthy individuals to indicate possible false-negatives
736  trait2<-
737  c(sort(trait[infected==F],decreasing=higher.healthy),trait[infected==T])
738
739  dist.reals<-1:Nperc
740  names(dist.reals)<-paste(as.character(percentages), "%")

```

```

741
742   contamination<-paste(as.character(percentages), "%")
743   names(contamination)<-paste(as.character(percentages), "%")
744
745   mean.healthy<-dist.reals
746   mean.infected<-dist.reals
747
748   sd.healthy<-dist.reals
749   sd.infected<-dist.reals
750
751   N.healthy<-dist.reals
752   N.infected<-dist.reals
753
754   mean.dist.perm<-dist.reals
755   p.vals.perm<-dist.reals
756
757   #Compute group means in non-permuted sample
758   for(i in 1:Nperc){
759     mean.healthy[i]<-mean(trait2[vector.ident[[i]]==F])
760     mean.infected[i]<-mean(trait2[vector.ident[[i]]==T])
761     sd.healthy[i]<-sd(trait2[vector.ident[[i]]==F])
762     sd.infected[i]<-sd(trait2[vector.ident[[i]]==T])
763     N.healthy[i]<-sum(vector.ident[[i]]==F)
764     N.infected[i]<-sum(vector.ident[[i]]==T)
765     dist.reals[i]<-abs((mean(trait2[vector.ident[[i]]==F]) -
766     mean(trait2[vector.ident[[i]]==T]))
767   }
768
769   cohen<-
770   abs(dist.reals)/((sd.healthy*N.healthy+sd.infected*N.infected)/(N.healthy+N
771   .infected))
772

```

```

773 #Skewness computation
774 skewness.healthy<-FPskewness(trait[infected==F])
775 skewness.infected<-FPskewness(trait[infected==T])
776
777 skew.diff<-abs(skewness.healthy-skewness.infected)
778
779 skewness<-c(skewness.healthy,skewness.infected)
780 skewness<-data.frame(skewness)
781
782 names(skewness)<-"Fisher-Pearson coefficient of skewness"
783 rownames(skewness)<-c("Identified as healthy","Identified as infected")
784
785 signs<-sign(dist.reals)
786
787 #Permutation test with skewness add-on
788 perm.dist<-array(NA,dim=c(Nperc,runs))
789 rand.skew<-NA
790
791 for(run in 1:runs){
792   trait2<-sample(trait)
793   rand.skew[run]<-abs(FPskewness(trait2[infected==F]) -
794     FPskewness(trait2[infected==T]))
795
796   trait2<-
797     c(sort(trait2[infected==F],decreasing=higher.healthy),trait2[infected==T])
798
799   for(i in 1:Nperc){
800     perm.dist[i,run]<-abs(mean(trait2[vector.ident[[i]]==F]) -
801       mean(trait2[vector.ident[[i]]==T]))
802   }
803 }
804

```

```

805 skew.p<-sum(rand.skew>skew.diff)/runs
806
807 skew.higher<-ifelse(skewness.healthy>skewness.infected,"test-
808 negative","test-positive")
809 skew.guess.higher<-ifelse(skewness.healthy>skewness.infected,FALSE,TRUE)
810 healthy.positive<-ifelse(skewness.healthy>0,TRUE,FALSE)
811 infected.positive<-ifelse(skewness.infected>0,TRUE,FALSE)
812 skew.sig<-ifelse(skew.p<0.05,TRUE,FALSE)
813
814 skew.message<-paste(
815   ifelse(healthy.positive==infected.positive,
816     paste(
817       "The distribution of trait value was",
818       ifelse(healthy.positive,"positively","negatively"),
819       "skewed \nin both groups.",
820       "The Fisher-Pearson coefficient of skewness \nwas higher
821       in",skew.higher,"group.")
822     ,
823     paste("The distribution of individuals identified as healthy \nwas skewed",
824       ifelse(healthy.positive,"positively","negatively"),
825       "the distribution of individuals \nidentified as infected",
826       ifelse(infected.positive,"positively","negatively."))
827   )
828   ,
829   paste("\n\nThe difference between the coefficients of skewness was",
830     ifelse(skew.sig," \nstatistically significant.\n"," \nhowever, not
831     statistically significant.\n"),
832     "(Two-tailed permutation test of skewness difference \non ",runs," runs was
833     executed.) ",sep="")
834   ,
835   ifelse(skew.sig==FALSE,
```

```

836 "\n\nThis might question the assumption of data contamination \nsince we
837 would expect a difference in skewness between \nthe groups in contaminated
838 data. \nProceed with caution.",

839 paste("\n\nThis supports the assumption of data contamination.",

840 "\nBased on the difference in skewness we would assume \ncontamination of
841 healthy group by false negative \nsubjects from the",

842 ifelse(skew.higher=="test-positive","lower","upper"),

843 "tail of the distribution \nof infected individuals, which would lead to
844 overall",

845 ifelse(skew.higher=="test-positive","\ndecrease","\nincrease"),

846 "of test-negative group mean.))

847 ,

848 ifelse(skew.sig==FALSE,"",

849 paste(ifelse(set.higher==skew.guess.higher,

850 paste("\n\nThe skewness analysis brings further support to the hypothesis
851 \nof",

852 ifelse(set.higher,"higher","lower"),

853 "mean in non-contaminated group of healthy \nindividuals, which was used in
854 current permutation test \nfor contaminated data.\n"),

855 paste("\n\nThe skewness analysis, however, does not support the hypothesis
856 \nof",

857 ifelse(set.higher,"higher","lower"),

858 "mean in non-contaminated group of healthy \nindividuals, which was used in
859 current permutation test \nfor contaminated data. Proceed with caution.\n")

860 )))

861 )

862

863 skewness<-rbind(skewness[1], "", skew.p)

864

865 rownames(skewness)[c(3,4)]<-c("", "p-value")

866

867 skewness[c(1,2,4),1]<-format(round(as.numeric(skewness[c(1,2,4),1]),3))

868

869 mean.dist.perm<-rowMeans(perm.dist)

```

```

870
871   for(i in 1:Nperc){
872     p.vals.perm[i]<-sum(dist.reals[i]<perm.dist[i,])/runs
873   }
874
875   mean.healthy<-format(round(mean.healthy,2))
876   mean.infected<-format(round(mean.infected,2))
877   sd.healthy<-format(round(sd.healthy,2))
878   sd.infected<-format(round(sd.infected,2))
879   cohen<-format(round(cohen,2))
880   dist.reals<-format(round(dist.reals,2))
881
882   mean.dist.perm<-format(round(mean.dist.perm,2))
883   p.vals.perm<-format(round(p.vals.perm,3))
884
885   res.table<-
886   rbind(contamination,mean.healthy,mean.infected,dist.reals,mean.dist.perm,sd
887   .healthy,sd.infected,N.healthy,N.infected,cohen,p.vals.perm)
888   res.table<-as.data.frame(res.table)
889
890   colnames(res.table)<-NULL
891   rownames(res.table)<-c("contamination","non-infeceted mean","infected
892   mean","mean difference","expected difference","non-infeceted SD","infected
893   SD","non-infeceted N","infected N","Cohen's d","p-value")
894
895   final.message<-paste("\nTwo-tailed permutation test for contaminated data
896   was executed. \nIt was assumed that",
897   ifelse(set.higher==T,"healthy","infected"),
898   "individuals have on average higher \ntrait value if all false negative
899   individuals are relocated correctly.")
900
901   cat("\nSample characteristics:\n")
902   print(orig.means)

```

```

903   cat(paste("\n",higher.report,"\n\n",sep=""))
904   cat(set.report1)
905
906   if(skewness.analysis==T){
907     cat("\n\nSkewness report:\n")
908     print(skewness)
909     cat("\n")
910     cat(skew.message)
911   }
912
913   cat("\n\nPermutation test for contaminated data:\n")
914   cat(paste(set.report2,"\n\n",sep=""))
915   cat(which.test)
916   cat(paste("\n",runs,"sample permutations were performed.\n"))
917   print(res.table)
918   cat(paste(final.message,"\n\n",sep=""))
919
920   results<-
921   list(orig.means,higher.report,set.report1,skewness,skew.message,set.report2
922   ,which.test,res.table,final.message)
923
924   return(invisible(results))
925 }
926

```


927 **Supplement 2**

928 **Skewness analysis**

```

929 #####
930 #####hit ctrl+a and ctrl+r to install the function####
931 #####
932
933 #This script contains skewness_comparison() function
934 #skewness_comparison(trait,identification,percentages=seq(0,50,1),higher.healthy=
935 (mean(trait[identification==F])>mean(trait[identification==T])),runs=
936 10000)
937
938 #Arguments are described below.
939 #It is necessary to define function calculating skewness index first:
940
941 ##Function that returns Fisher-Pearson coefficient of skewness.
942 ##Input is a vector of numerical values.
943
944 FPSkewness<-function(x){
945   return((sum((x-mean(x))^3)/length(x))/((sqrt(sum((x-
946   mean(x))^2)/length(x))^3))
947 }
948
949 ###Skewness comparison
950 ###Function that reports how relocation of seronegative individuals changes
951 the skewness of the distribution in both seronegative and seropositive
952 groups.
953 ###trait - Numerical vector of trait values
954 ###identification - Logical vector of assumed presence (T) or absence (F)
955 of infection
956 ###percentages - Numerical vector of percentages of false negative amongst
957 negative subjects (contamination levels) for which the permutation test for
958 contaminated data will be run
959 ###higher healthy - Logical. Indicates whether we assume the healthy
960 individuals to show higher (T) or lower (F) trait values. When not

```

```

961 specified, the script assumes this relationship based of group means with
962 no hypothesised contamination.

963 #####It allows us to use the difference between the groups (not in
964 absolute values) in permutation test. In this scenario the seropositive
965 group mean is substracted from seronegative group mean and the one-tailed
966 permutation test is conducted accordingly.

967 ###runs - Number of resamplings used in permutation test
968
969 skewness_comparison<-
970 function(trait,identification,percentages=seq(0,50,1),higher.healthy=(mean(
971 trait[identification==F])>mean(trait[identification==T])),runs=10000){
972
973   if(length(trait)!=length(identification)){
974     stop("The vectors of trait values and infection indication are of different
975     lengths.")
976   }
977
978   higher<-(mean(trait[identification==F])>mean(trait[identification==T]))
979   set.higher<-higher.healthy
980
981   orig.means<-tapply(trait,identification,mean)
982   orig.means<-data.frame(orig.means)
983
984   names(orig.means)<-"Original mean values"
985   rownames(orig.means)[which(rownames(orig.means)=="FALSE")]<-"Identified as
986   healthy"
987   rownames(orig.means)[which(rownames(orig.means)=="TRUE")]<-"Identified as
988   infected"
989
990   higher.report<-ifelse(higher==T,
991     "In original sample, individuals identified as healthy showed higher
992     \naverage trait value.",
993     "In original sample, individuals identified as infected showed higher
994     \naverage trait value."
995   )

```

```

996
997 concord<-ifelse(higher==set.higher,"Consequently","Despite that,")
998
999 set.report1<-paste(concord,ifelse(set.higher==T,
1000 "healthy individuals were hypothesised to have higher \naverage trait value
1001 in a contamination-free sample. \n",
1002 "infected individuals were hypothesised to have higher \naverage trait
1003 value in a contamination-free sample. \n"
1004 ))
1005
1006
1007 run.report<-paste("Two-tailed permutation test of skewness difference \non
1008 ",runs," runs was executed on each contamination level.\n\n",sep="")
1009
1010 set.report2<-paste("\nFor each contamination level respective proportion of
1011 seronegative \nindividuals with",
1012 ifelse(set.higher==T,"lowest","highest"),
1013 "trait value was relabeled as seropositive \nand the difference between the
1014 group skewness was measured. \nReferential skewness differences from
1015 permutation runs were based \non random non-contaminated sample with group
1016 sizes corresponding \nto respective contamination levels.\n\n"
1017 )
1018
1019 trait<-c(trait[identification==F],trait[identification==T])
1020 infected<-sort(identification)
1021
1022 count.healthy<-sum(!identification)
1023 count.infected<-sum(identification)
1024
1025 Nperc<-length(percentages)
1026
1027 vector.ident<-list()
1028

```

```

1029   for(i in 1:Nperc){
1030     reassign<-round(count.healthy*(percentages[i]/100))
1031     identification<-c(rep(F,count.healthy-
1032       reassign),rep(T,count.infected+reassign))
1033     vector.ident[[i]]<-identification
1034   }
1035
1036   #Sorts healthy individuals to indicate possible false-negatives
1037   trait2<-
1038   c(sort(trait[infected==F],decreasing=higher.healthy),trait[infected==T])
1039
1040   skewness.healthy<-1:Nperc
1041   names(skewness.healthy)<-paste(as.character(percentages), "%")
1042   skewness.infected<-skewness.healthy
1043
1044   contamination<-paste(as.character(percentages), "%")
1045   names(contamination)<-paste(as.character(percentages), "%")
1046
1047   #Compute group means in non-permuted sample
1048   for(i in 1:Nperc){
1049     skewness.healthy[i]<-FPskewness(trait2[vector.ident[[i]]==F])
1050     skewness.infected[i]<-FPskewness(trait2[vector.ident[[i]]==T])
1051   }
1052
1053   skew.diff<-abs(skewness.healthy-skewness.infected)
1054
1055   #Permutation test of skewness difference
1056
1057   skew.diff.perm<-array(NA,dim=c(Nperc,runs))
1058
1059   for(run in 1:runs){

```

```

1060   trait2<-sample(trait)
1061
1062   for(i in 1:Nperc){
1063     skew.diff.perm[i,run]<-abs(FPskewness(trait2[vector.ident[[i]]==F]) -
1064     FPskewness(trait2[vector.ident[[i]]==T]))
1065   }
1066 }
1067
1068 p.vals.skew<-NA
1069
1070 for(i in 1:Nperc){
1071   p.vals.skew[i]<-sum(skew.diff[i]<skew.diff.perm[i,])/runs
1072 }
1073
1074 guess.perc<-percentages[which(skew.diff==min(skew.diff))]
1075
1076 possible<-percentages[p.vals.skew>0.05]
1077
1078 if(length(possible)==0){
1079   ok.report<-paste("\nThe difference in Fisher-Pearson coefficients of
1080   skewness between \ngroups was significant at all investigated contamination
1081   levels.\nThis may be caused by extreme proportion of false negative
1082   \nindividuals, insufficient number of relocated fractions, \ndifferent
1083   shapes of distributions of healthy and infeceted \nindividuals, or, most
1084   likely, by wrong setting of higher group \nmean in non-contaminated sample.
1085   \nTry running this comparison with parameter higher.healthy
1086   =",ifelse(higher.healthy==TRUE,"FALSE","TRUE"), "\n\n")
1087 }else{
1088   if(min(possible)==max(possible)){
1089     ok.report<-paste("\nThe difference in Fisher-Pearson coefficients of
1090     skewness between \ngroups was not significant at",
1091     min(possible),
1092     "% of relocated individuals.\n\n")
1093   }else{

```

```

1094 ok.report<-paste("\nThe difference in Fisher-Pearson coefficients of
1095 skewness between \ngroups was not significant between",
1096 min(possible),
1097 "% and",
1098 max(possible),
1099 "% of relocated individuals.\n\n")
1100 }
1101 }
1102
1103 best.guess<-paste("The difference between group skewness was smallest
1104 \nwhen",
1105 guess.perc,
1106 "% of seronegative individuals with",
1107 ifelse(higher.healthy,"lowest","highest"),
1108 "trait value \nwas relocated to seropositive group.\n\n")
1109
1110
1111 skewness.healthy<-format(round(skewness.healthy,2))
1112 skewness.infected<-format(round(skewness.infected,2))
1113 p.vals.skew<-format(round(p.vals.skew,3))
1114
1115 skewness.res<-
1116 rbind(contamination,skewness.healthy,skewness.infected,"",p.vals.skew)
1117
1118 skewness.res<-as.data.frame(skewness.res)
1119
1120 colnames(skewness.res)<-NULL
1121 rownames(skewness.res)<-c("contamination","skewness healthy","skewness
1122 infected","", "p-value")
1123
1124
1125 cat("\nSample characteristics:\n")

```

```

1126   print(orig.means)
1127   cat(paste("\n",higher.report,"\n\n",sep=""))
1128   cat(set.report1)
1129   cat(set.report2)
1130
1131   cat(run.report)
1132
1133   cat(paste("Skewness comparison:", "\n", sep=""))
1134
1135   print(skewness.res)
1136
1137   cat(ok.report)
1138   cat(best.guess)
1139
1140   results<-list(orig.means,higher.report,set.report1,skewness.res)
1141
1142   return(invisible(results))
1143 }
1144

```

1145 **Supplement 3**

1146 **Example**

1147 #####

1148 ###Exemplar runs###

1149 #####

1150

1151 #Both functions contamination_perm_test() and skewness_comparison() must be
1152 installed, run respective scripts

1153

1154 #You can generate data with known proportion of false negative individuals
1155 with this script and try permutation test for contaminated data on them.

1156

1157 count<-1000 #sets sample size

1158 inf.prop<-0.5 #sets proportion of seropositive individuals

1159 fixed.effect<-(-3) #sets the effect of infection on simulated trait

1160 healthy.average<-101.5 #sets the average trait value in noninfected group

1161 sd<-12 #sets the standard deviation of within group

1162

1163 false.negatives<-5 #sets proportion of false negative individuals

1164

1165 ###Computes counts in respektive groups using set properties

1166 count.infected<-round(inf.prop*count)

1167 count.healthy<-count-count.infected

1168

1169 ###Creates a variables that indicates infection

1170 infected<-c(rep(F,count.healthy),rep(T,count.infected))

1171

1172 #Calculates how many false negative individuals will be in the seronegative
1173 group

1174 reassign<-round(count.healthy*(false.negatives/100))

1175 #Creates a vector of actual iinfection


```

1176 really.infected<-c(rep(F,count.healthy-
1177 reassign),rep(T,count.infected+reassign))
1178
1179 #Generates the population (all healthy individuals)
1180 trait<-rnorm(count,healthy.average,sd)
1181
1182 #modifies really infected individuals
1183 trait[(sum(!really.infected)+1):count]<-
1184 trait[(sum(!really.infected)+1):count]+fixed.effect
1185
1186 #sorts really infected individuals such that most changed ones are close
1187 in the vector to healthy ones i.e. are marked as test-negative
1188 trait<-
1189 c(trait[really.infected==F],sort(trait[really.infected==T],decreasing=(sign
1190 (fixed.effect)==1)))
1191
1192 #scrambles the vectors, along the same random vector
1193 scramble<-sample(1:count)
1194
1195 infected<-infected[scramble]
1196 trait<-trait[scramble]
1197
1198 #####
1199 ###Trial data are ready###
1200 #####
1201
1202 #Executes permutation test for contaminated data with default argument
1203 values
1204 contamination_perm_test(trait,infected)
1205
1206 #Executes permutation test for contaminated data and skewness analysis
1207 contamination_perm_test(trait,infected,skewness.analysis=T)
1208

```

```

1209 #Executes permutation test for contaminated data, hypothesised difference is
1210 specified by hand. This comes useful when you have a reason to suspect
1211 paradoxical switch in group means.

1212 contamination_perm_test(trait,infected,higher.healthy=F)
1213

1214 #Executes permutation test on only 100 permutation runs per test - it is
1215 quicker, but less accurate

1216 contamination_perm_test(trait,infected,runs=100)
1217

1218 #Executes two tailed permutation test for contaminated dat
1219 contamination_perm_test(trait,infected,two.tailed=T)
1220

1221 #Executes skewness comparison to estimate proportion and distribution tail
1222 of possible contamination prior to the test

1223 skewness_comparison(trait,infected)
1224

1225 #Executes permutation test for contaminated data, levels of contamination
1226 are specified by hand

1227 contamination_perm_test(trait,infected,percentages=seq(1,15,1))

```