G-TWYST
GM PLANTS TWO YEAR SAFETY TESTING

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Cross studies statistical comparisons of NK33- and NK33+ versus the control feed

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G-TwYST (EU grant agreement no: 632165)

Biometris report 38.03.18 March 2018

https://doi.org/10.18174/455293

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March 2018

1 Introduction

Statistical analysis of G-TwYST studies A, B and C were reported separately in the following reports:

Goedhart, P.W. & van der Voet, H. (2017). G TwYST Study B. a 90-day toxicity study in rats fed GM maize NK603. Statistical report. Report 31.10.17, Biometris, Wageningen, The Netherlands.

Goedhart, P.W. & van der Voet, H. (2018). G TwYST Study A. Combined chronic toxicity and carcinogenicity study in rats fed GM maize NK603. Main statistical report. Report 32.02.18, Biometris, Wageningen, The Netherlands.

Goedhart, P.W. & van der Voet, H. (2018). G TwYST Study A. Combined chronic toxicity and carcinogenicity study in rats fed GM maize NK603. Statistical report, 3 months. Report 33.02.18, Biometris, Wageningen, The Netherlands.

Goedhart, P.W. & van der Voet, H. (2018). G TwYST Study A. Combined chronic toxicity and carcinogenicity study in rats fed GM maize NK603. Statistical report, 6 months. Report 34.02.18, Biometris, Wageningen, The Netherlands.

Goedhart, P.W. & van der Voet, H. (2018). G TwYST Study A. Combined chronic toxicity and carcinogenicity study in rats fed GM maize NK603. Statistical report, 12 months. Report 35.02.18, Biometris, Wageningen, The Netherlands.

Goedhart, P.W. & van der Voet, H. (2018). G TwYST Study A. Combined chronic toxicity and carcinogenicity study in rats fed GM maize NK603. Statistical report, 24 months. Report 36.02.18, Biometris, Wageningen, The Netherlands.

Goedhart, P.W. & van der Voet, H. (2018). G TwYST Study C. a 90-day toxicity study in rats fed GM maize NK603. Statistical report. Report 37.03.18, Biometris, Wageningen, The Netherlands.

Note that for Study A there are separate reports for data acquired after 3, 6, 12 and 24 months as well as a main report. Study B and C were 3 months studies only. The following five groups of endpoints were observed across studies and months:

1. Weights: final bodyweight, growth rate and mean of feed consumption. The growth rate obtained after three months was defined by an exponential curve, while the growth rates during the months 4- 6 and 7-12 month were obtained by linear regression. The growth rate during the month 13-24 in study A was not estimated because the weights had an irregular pattern.
2. Haematology: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYMR and LYMA
3. Clinical Biochemistry: ALP, ALT, AST, BIL, ALB, TP, Glu, CHOL, TAG, Crea, Urea, cHGB, Ca, Cl, K, Na and P
4. Organs weights expressed as percentage of final bodyweight: Kidney, Spleen, Liver, AdrenGl, Heart, Thymus and Brain. In addition for males Testis and Epididymis, and for females Ovary and Uterus. These endpoints were only obtained in studies B and C, i.e. after 3 months, and in study A after 12 months.
5. Urine: uVol, uVolW, uLeu, uOsmoll, uKeton and upH

In all three studies feeds with 33% inclusion rate of the near isogenic maize and the GM NK603 maize, without and with roundup, were used. The latter two feeds were coded as NK33- and NK33+. In all reports the GM feeds were compared to the non-GM control feed and a 95% confidence interval for the ratio was reported for all endpoints listed above. In this report these 95% confidence

intervals are graphically displayed side-by-side such that a quick comparison can be made. The confidence interval are coded by "*B-03*" and "*C-03*" for study B and C, which emphasizes that these are 3 months studies, and by "*A-03*", "*A-06*", "*A-12*" and "*A-24*" for study A. While assessing these figures it should be kept in mind that

1. The four confidence interval intervals for study A, i.e. for 3, 6, 12 and 24 months, are not independent since they are based on the same animals;
2. For the study A 24 month data only data for those animals that survived for two years were statistically analysed;
3. The confidence intervals employed the residual variance and the associated degrees of freedom of a simultaneous analysis of variance of all feeds;
4. Intervals are based on various numbers of replication, see Table 1;
5. The Urine endpoints uLeu and uKeton only attained a limited set of values, and an analysis of variance might therefore not be appropriate;
6. For intervals that do not encompass the value 1 the corresponding estimate is coloured fuchsia. For such endpoints there is a significant difference at the 5% level between the GM feed and the control feed.

Table 1 Number of cages per feeding group classified by study and endpoint group. The number of cages for Study A after 24 month is variable due to animals that died before the end of the study.

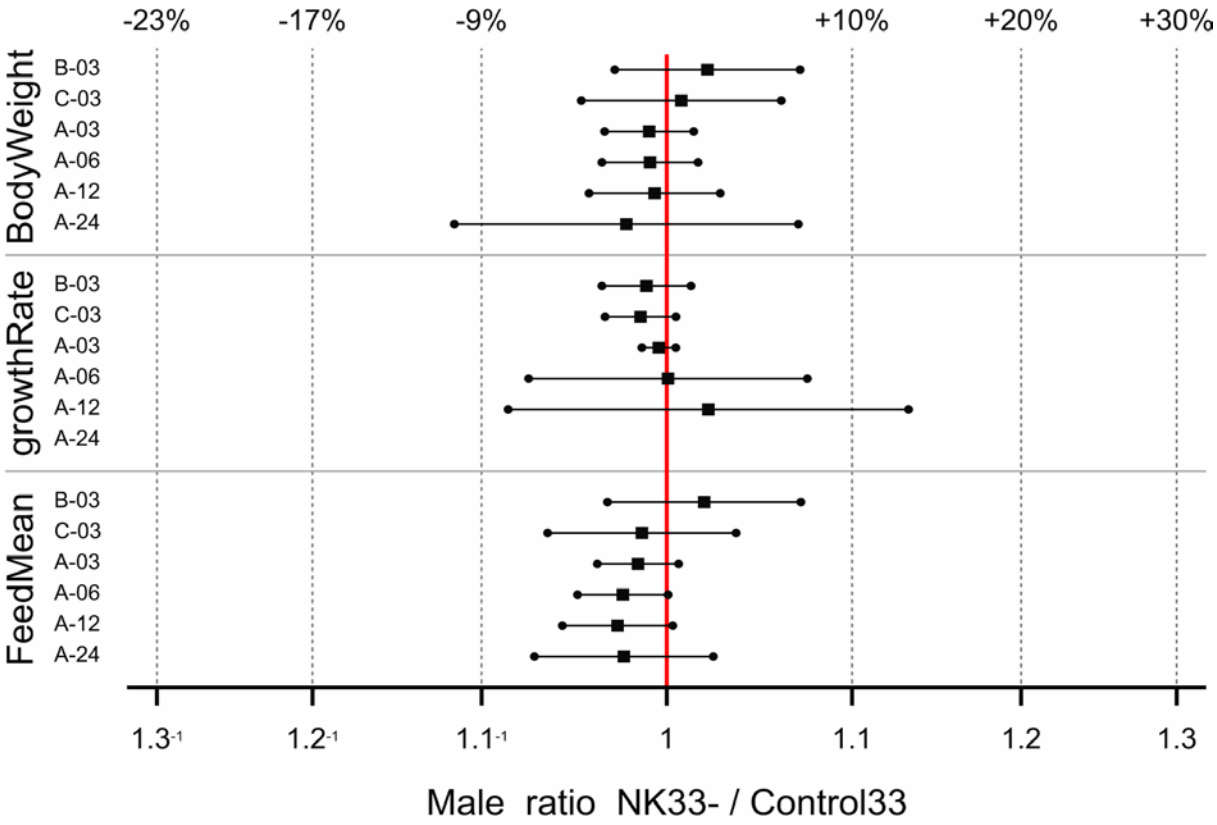
Endpoint group	B-03	C-03	A-03	A-06	A-12	A-24
Weights	8	8	35	35	35	17 – 23
Haematology	8	8	20	20	20	15 – 23
Clinical Biochemistry	8	8	20	20	20	16 – 23
Organs	8	8	-	-	10	-
Urine	8	8	10	10	15	15 - 20

In addition the coefficient of variation CV, as estimated from the residual variance σ^2 of the analysis of variance on the log-transformed data, is graphically displayed for every endpoint and every study. The coefficient of variation, expressed as a percentage, is calculated by means of

$$CV = 100\left(\sqrt{\exp(\sigma^2) - 1}\right).$$

In general, see Figure 23 to Figure 33, the CV is largest for data acquired after 24 months in study A, while for the other studies there is not a clear pattern for the endpoints considered in this report.

2 List of Figures



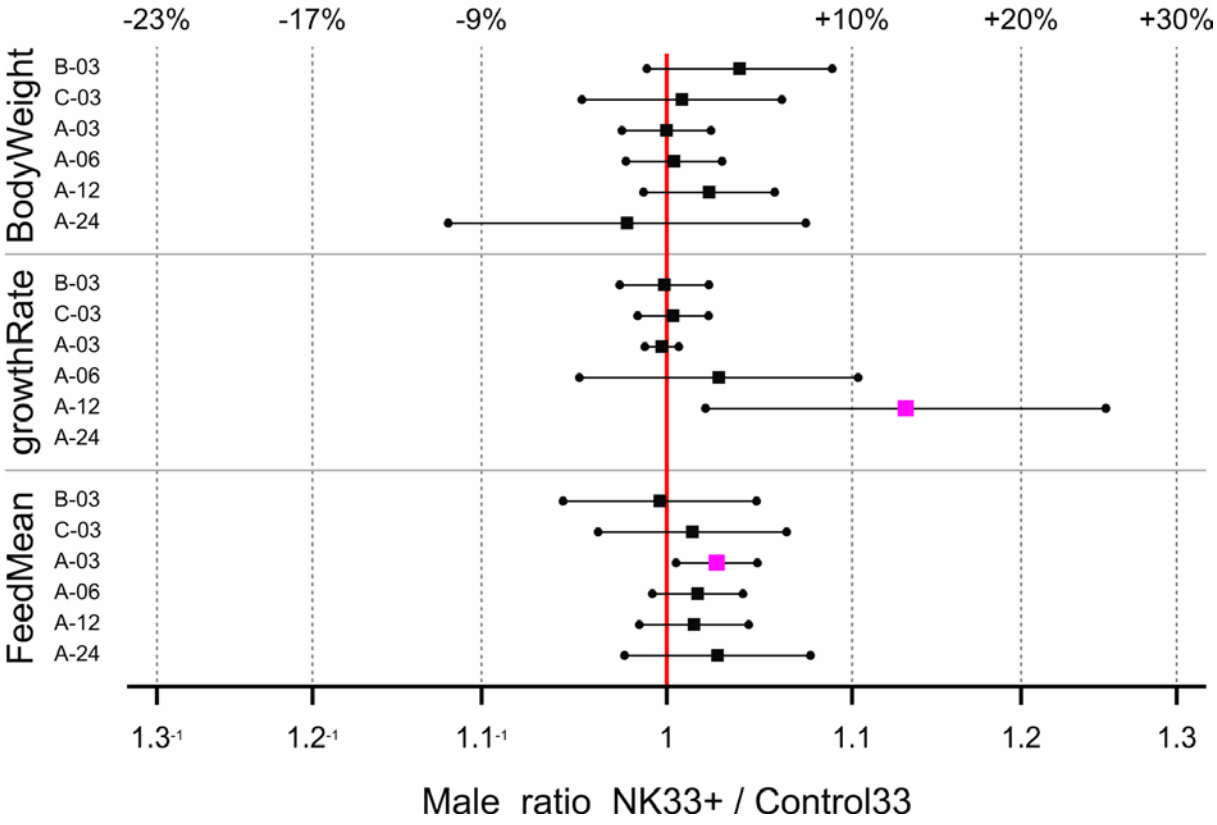
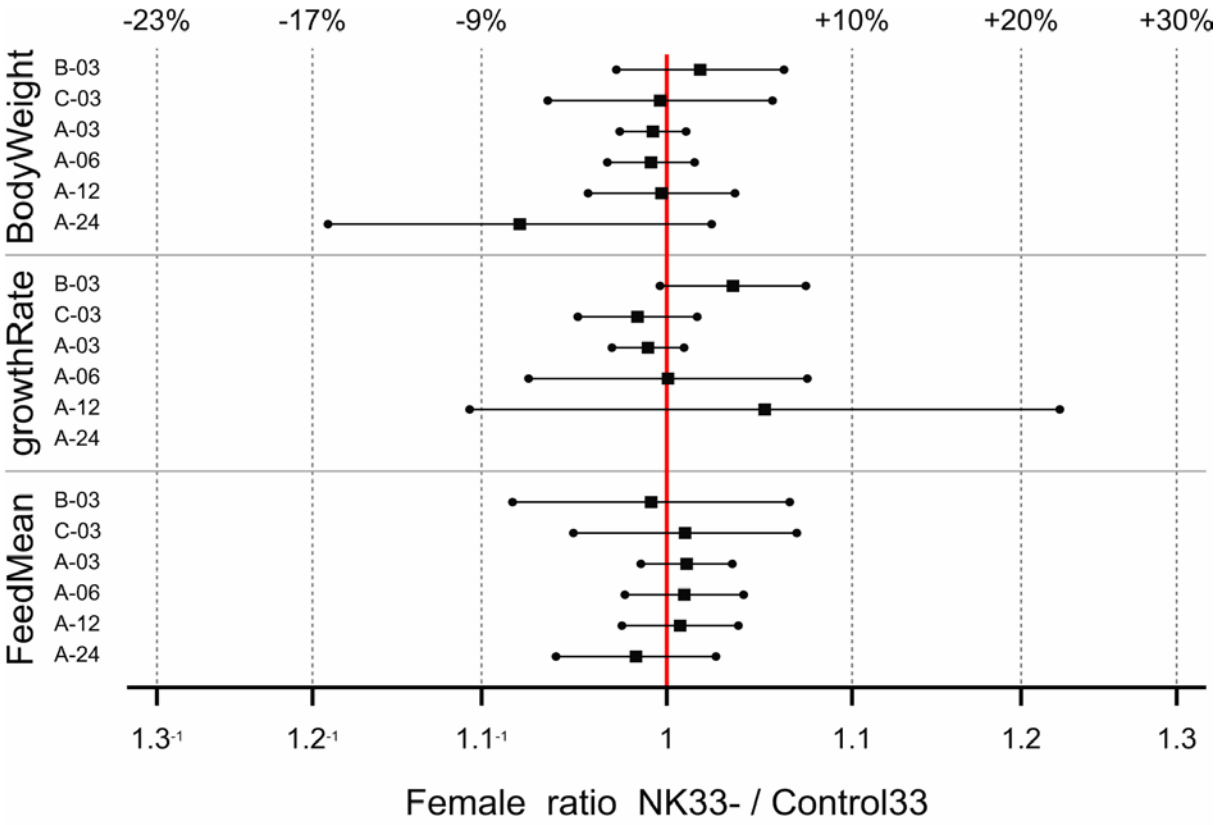


Figure 1 95% confidence intervals for Weights endpoints for males.



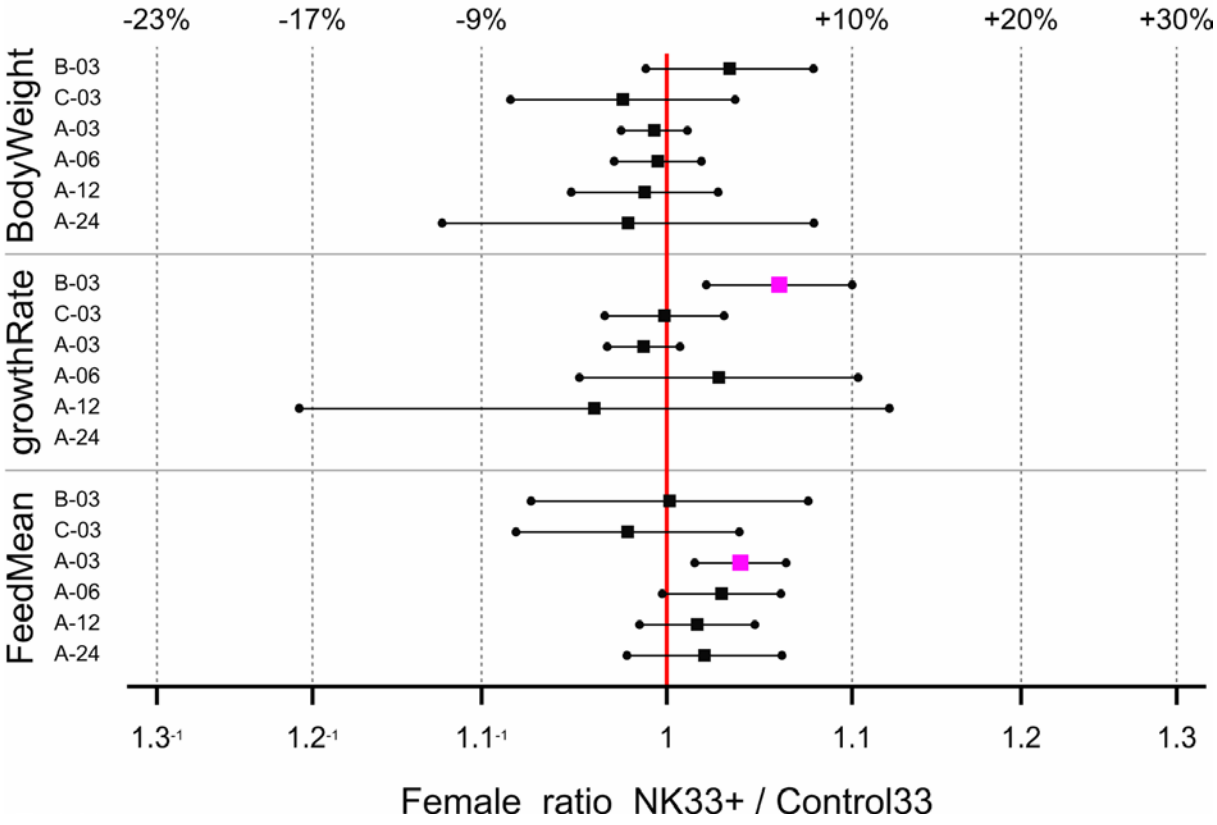


Figure 2 95% confidence intervals for Weights endpoints for females.

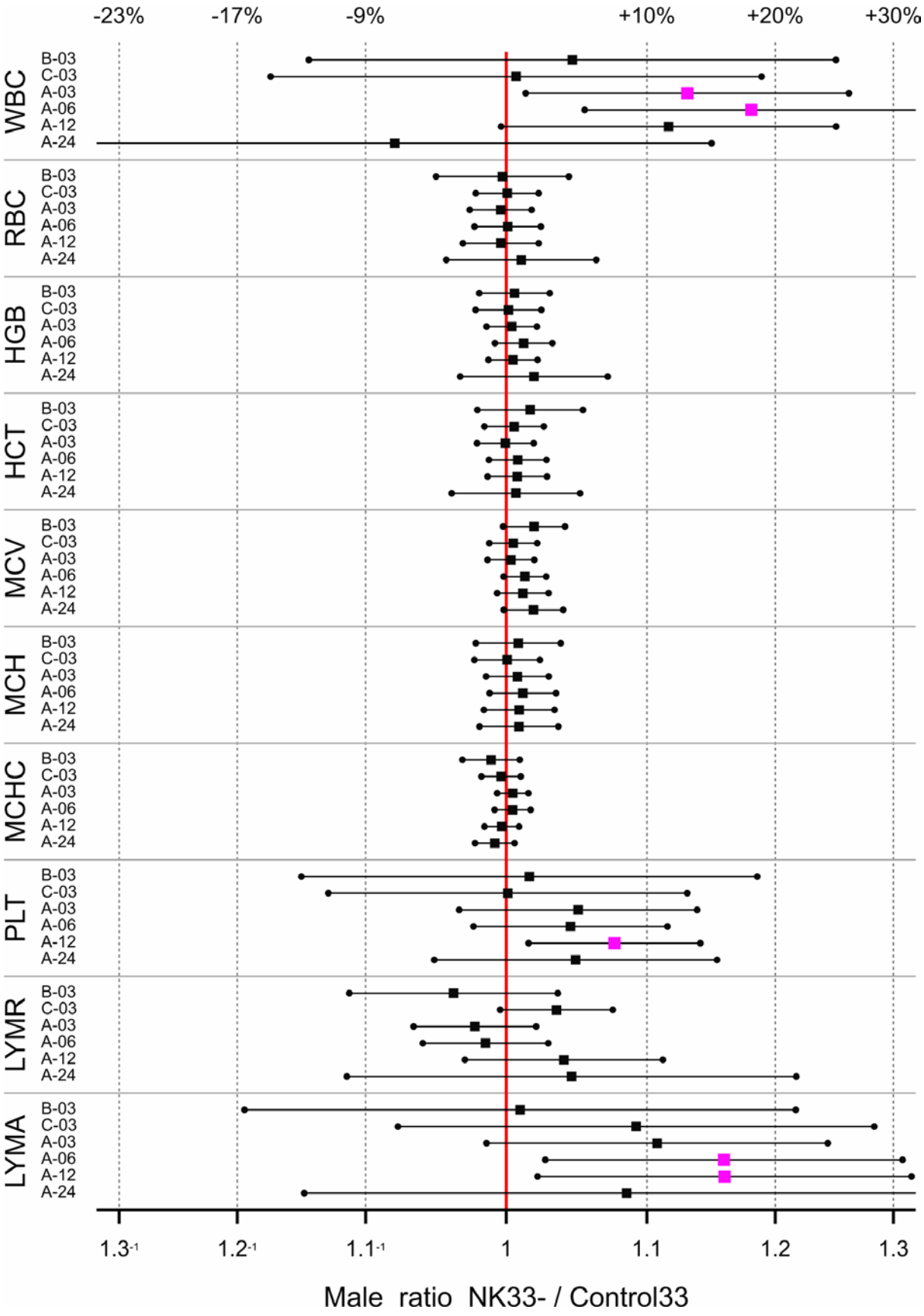


Figure 3 95% confidence intervals for Haematology endpoints for NK33- / Control for males.

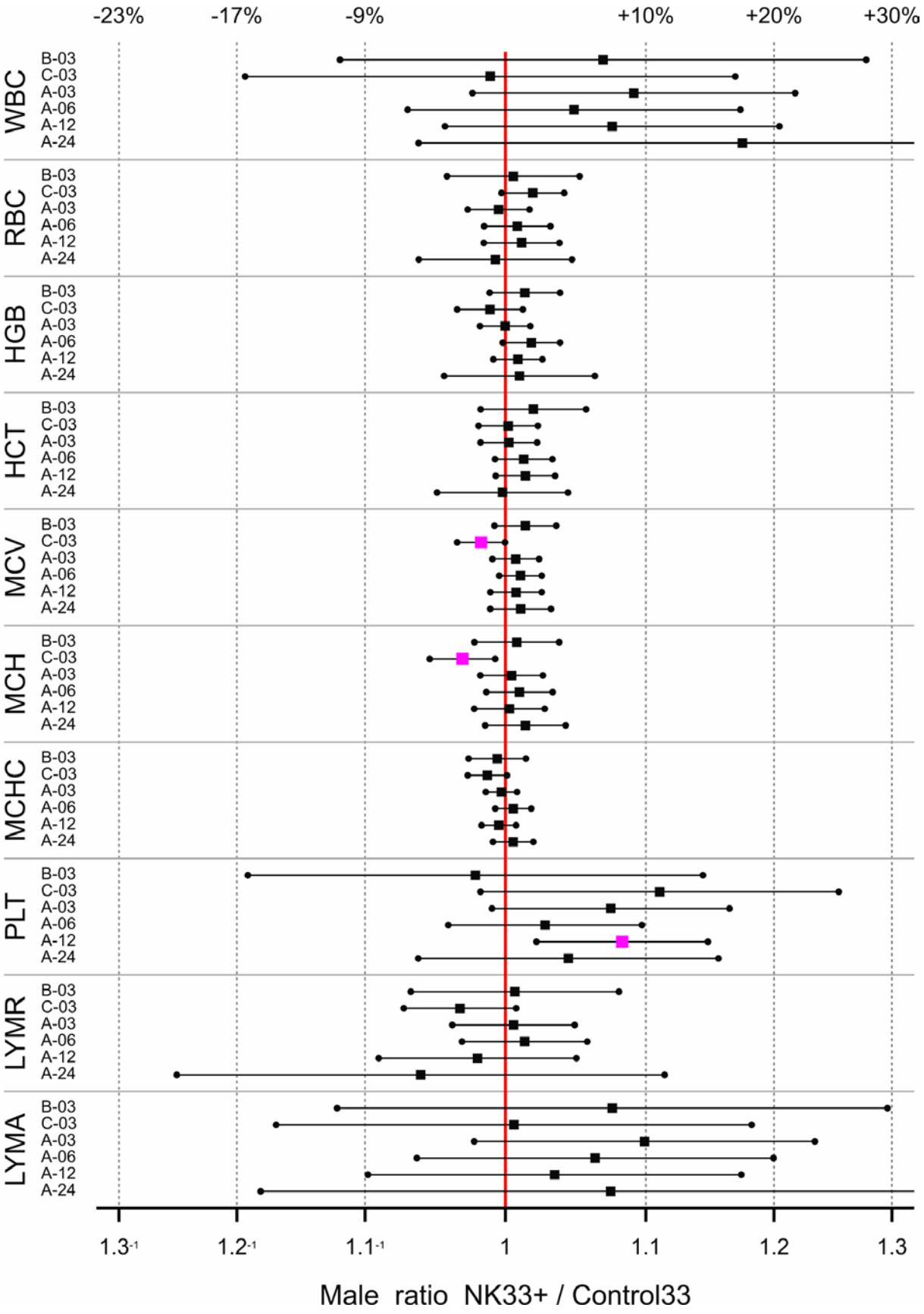


Figure 4 95% confidence intervals for Haematology endpoints for NK33+ / Control for males.

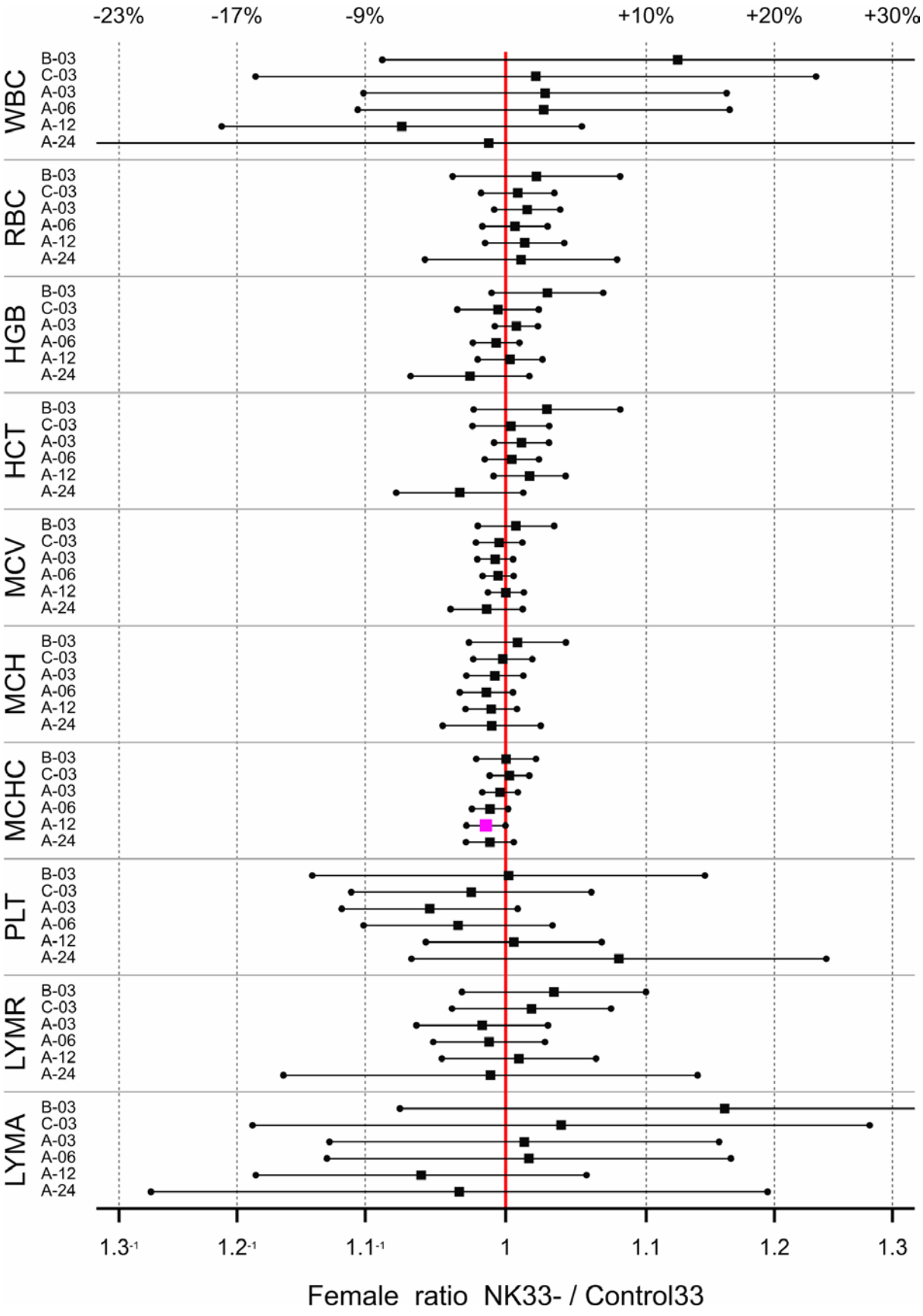


Figure 5 95% confidence intervals for Haematology endpoints for NK33- / Control for females.

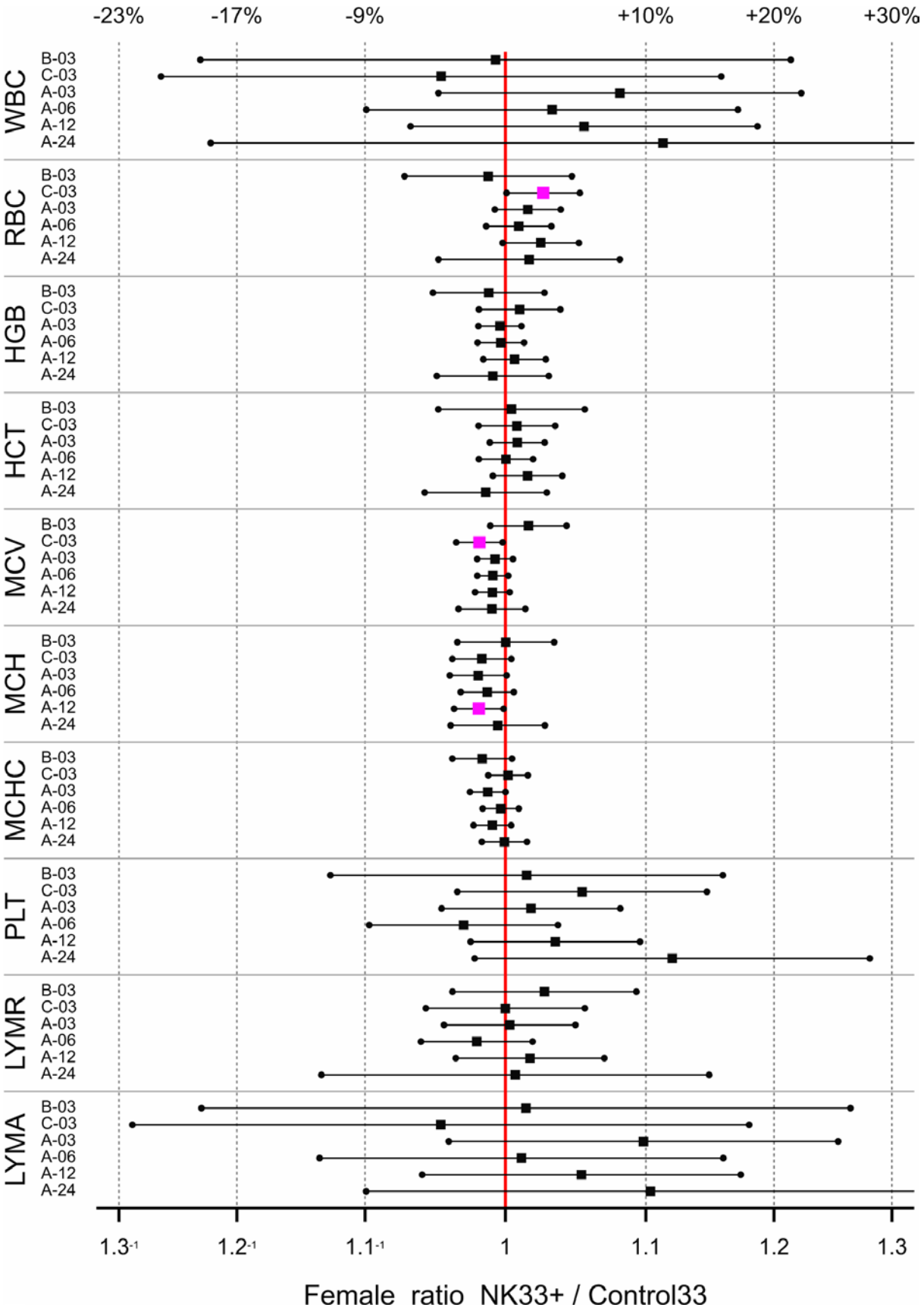


Figure 6 95% confidence intervals for Haematology endpoints for NK33+ / Control for females.

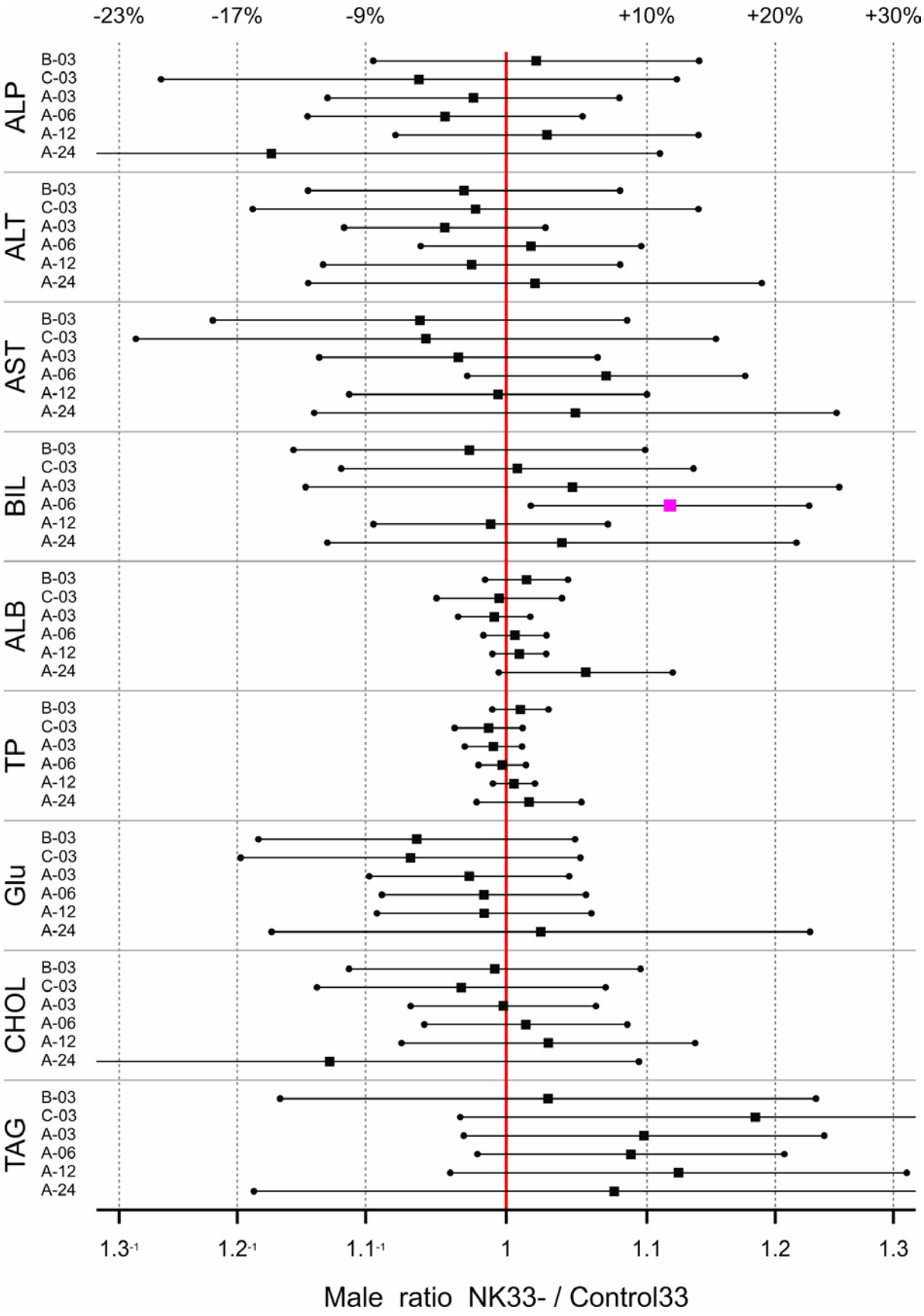


Figure 7 95% confidence intervals for Clin. Biochem. endpoints for NK33- / Control for males.

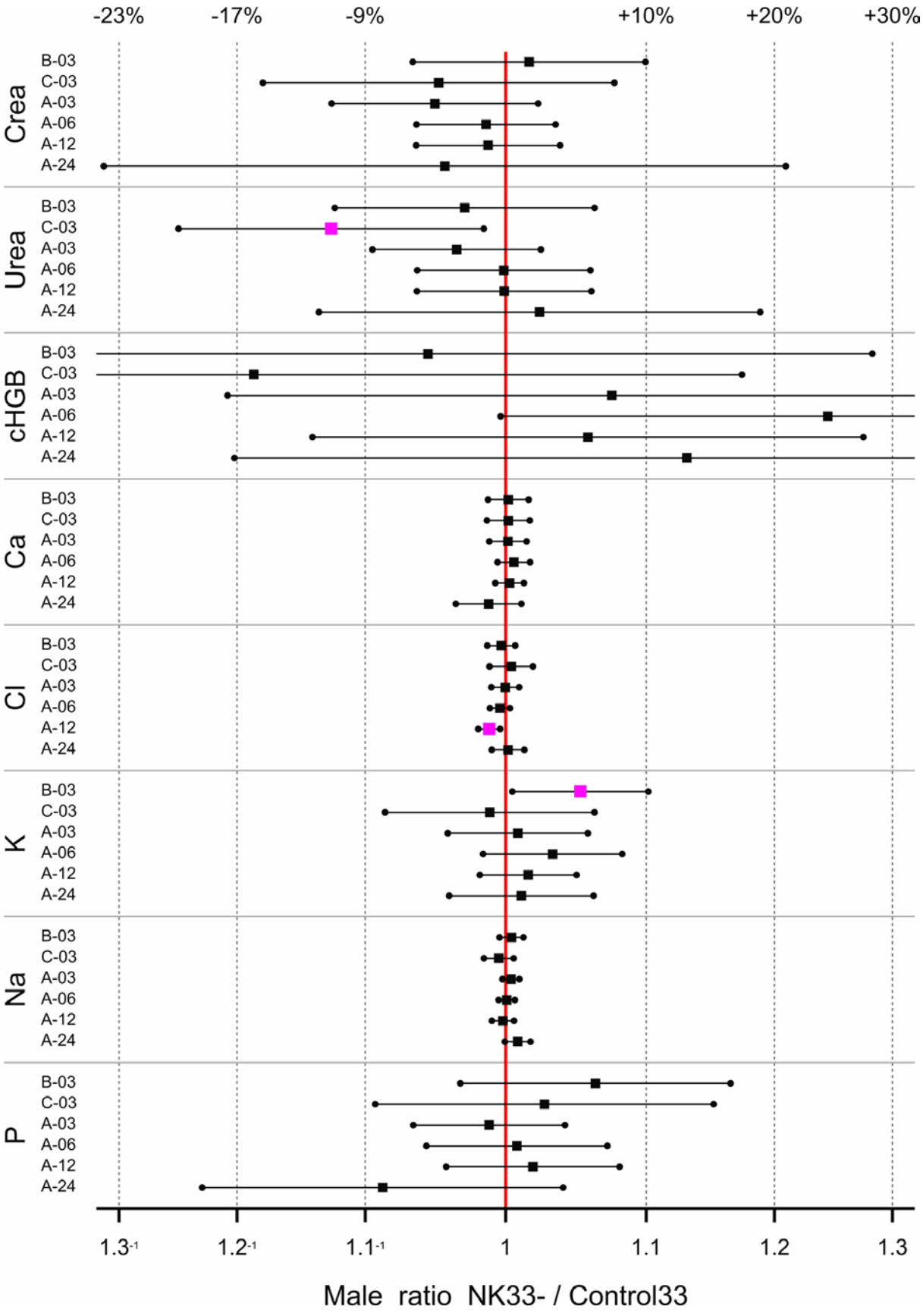


Figure 8 95% confidence intervals for Clin. Biochem. endpoints for NK33- / Control for males.

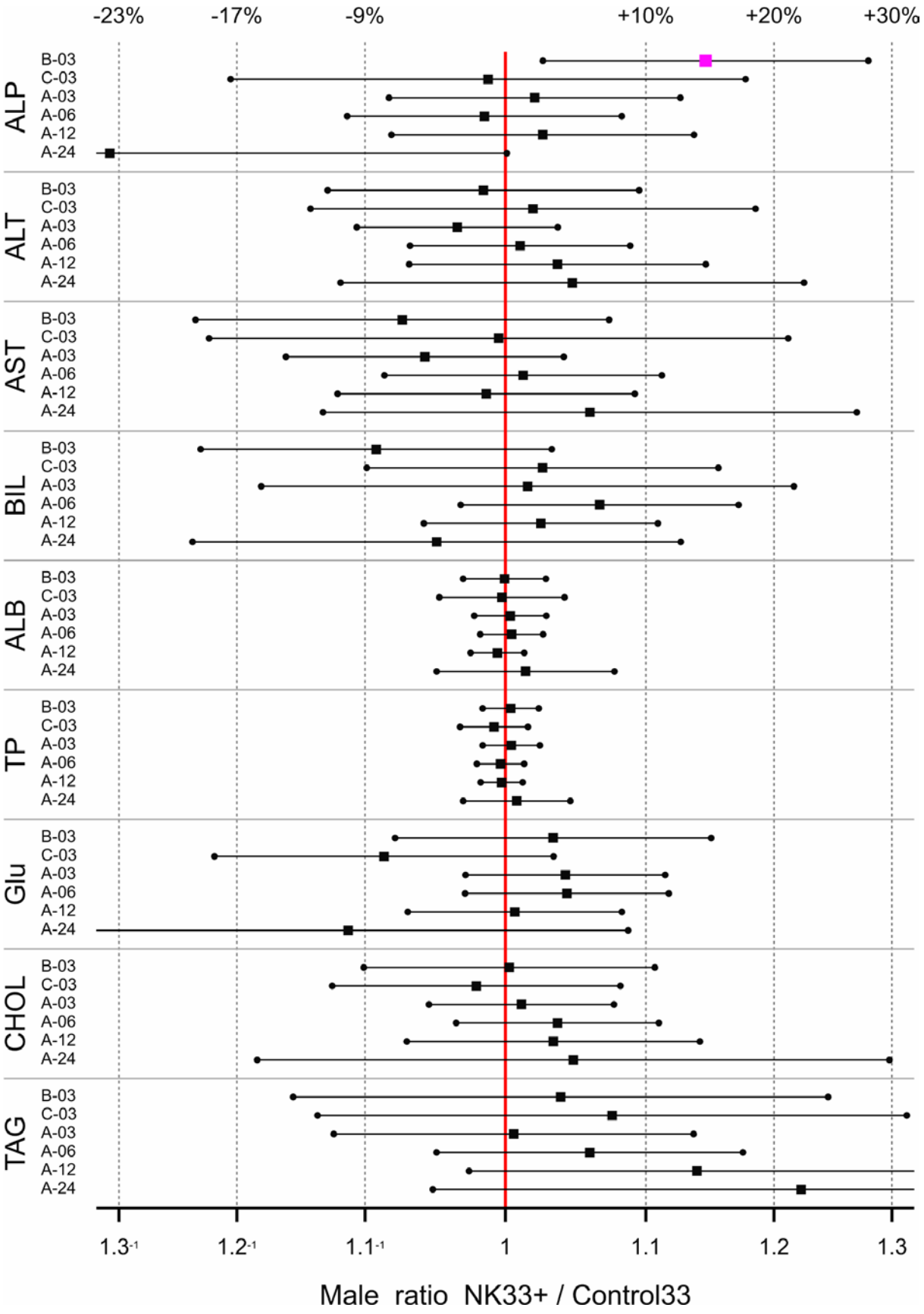


Figure 9 95% confidence intervals for Clin. Biochem. endpoints for NK33+ / Control for males.

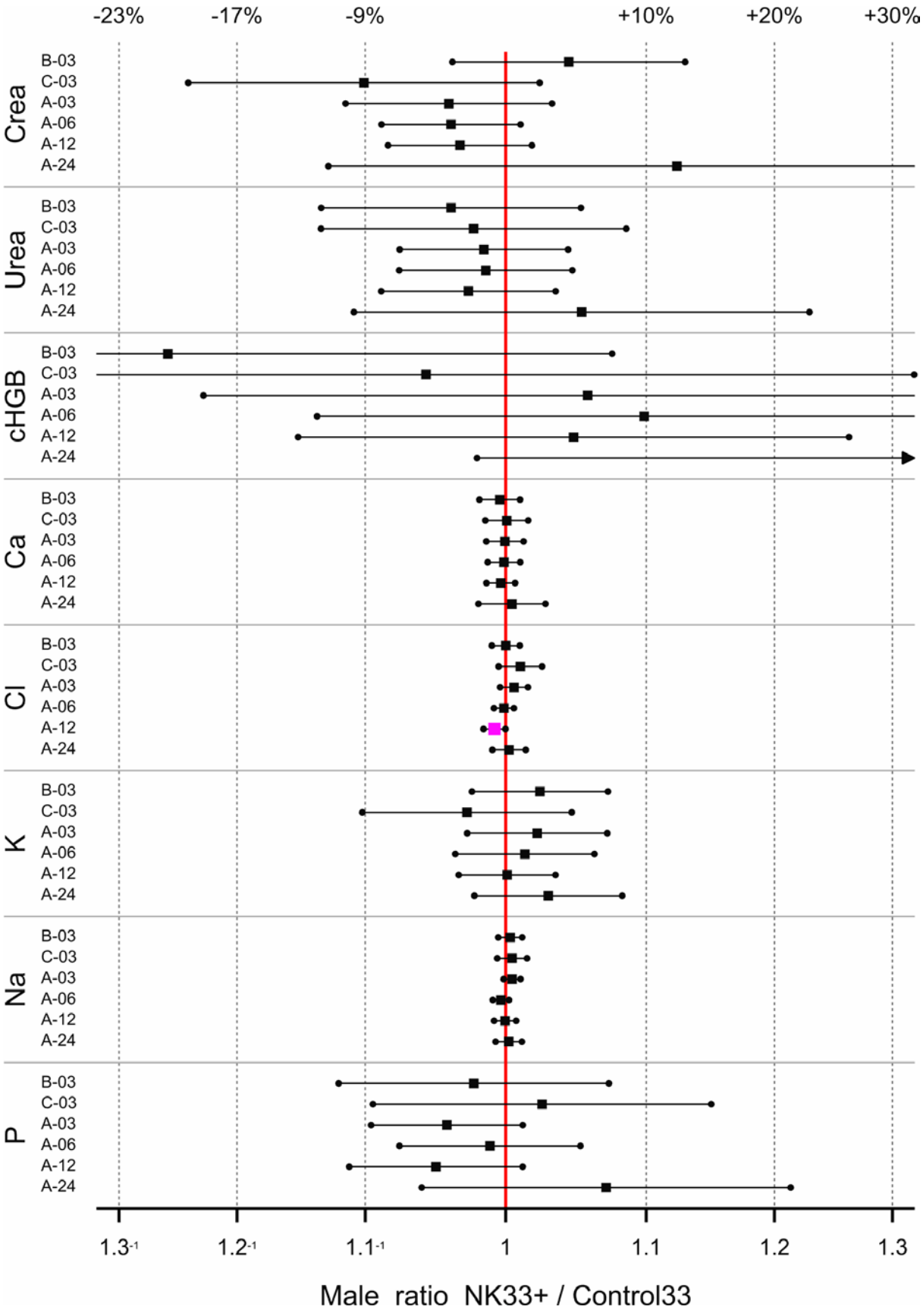


Figure 10 95% confidence intervals for Clin. Biochem. endpoints for NK33+ / Control for males.

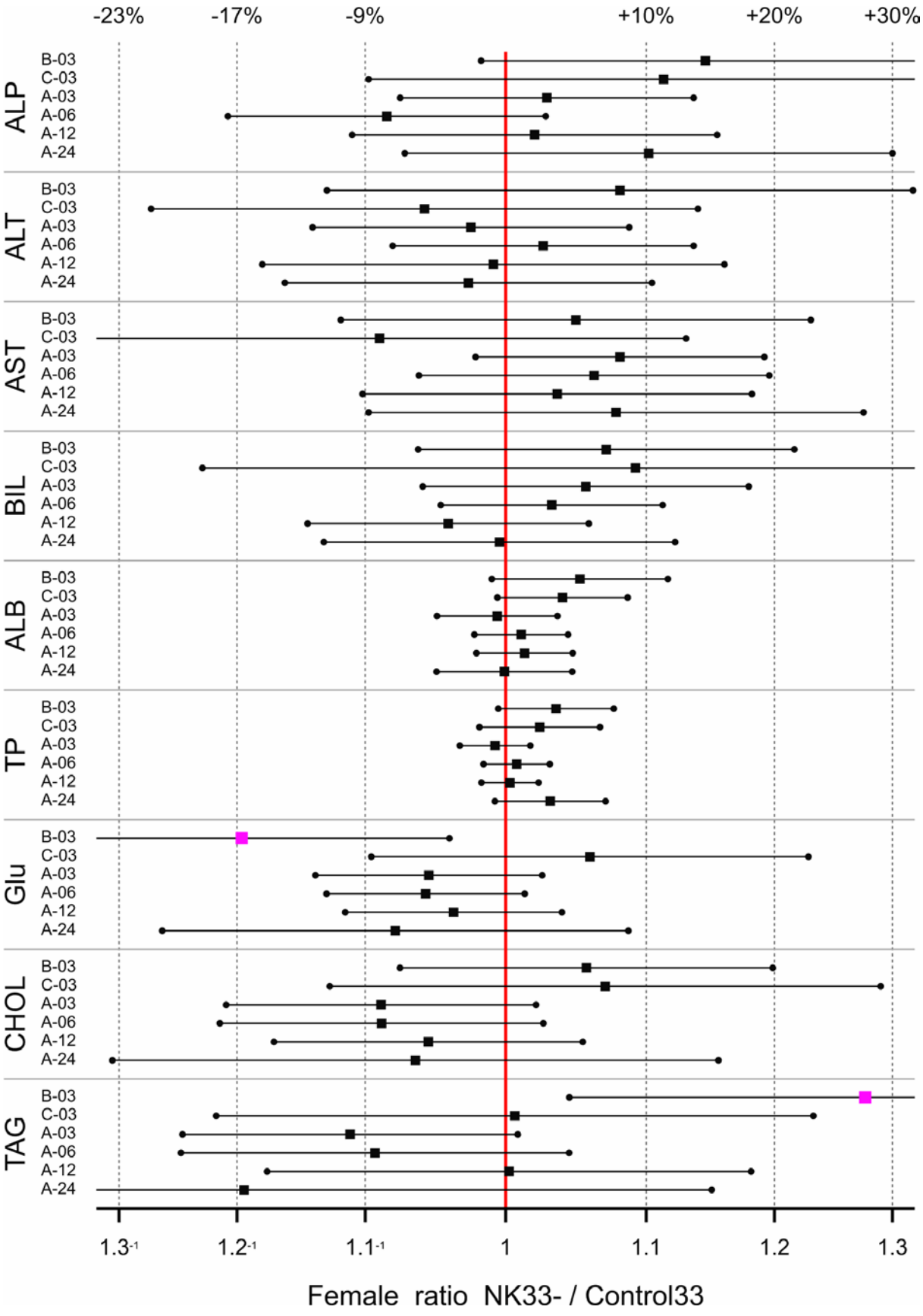


Figure 11 95% confidence intervals for Clin. Biochem. endpoints for NK33- / Control for females.

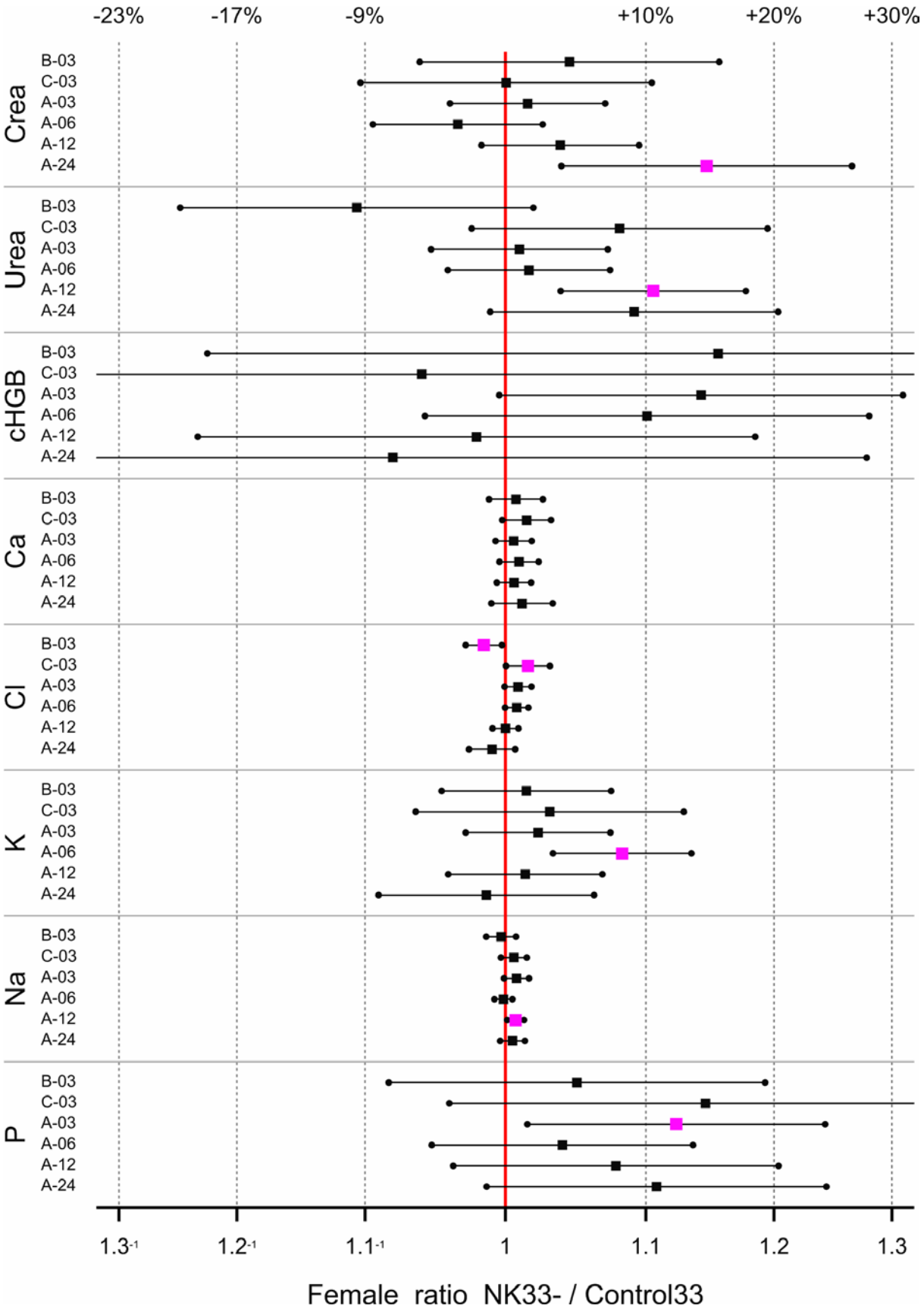


Figure 12 95% confidence intervals for Clin. Biochem. endpoints for NK33- / Control for females.

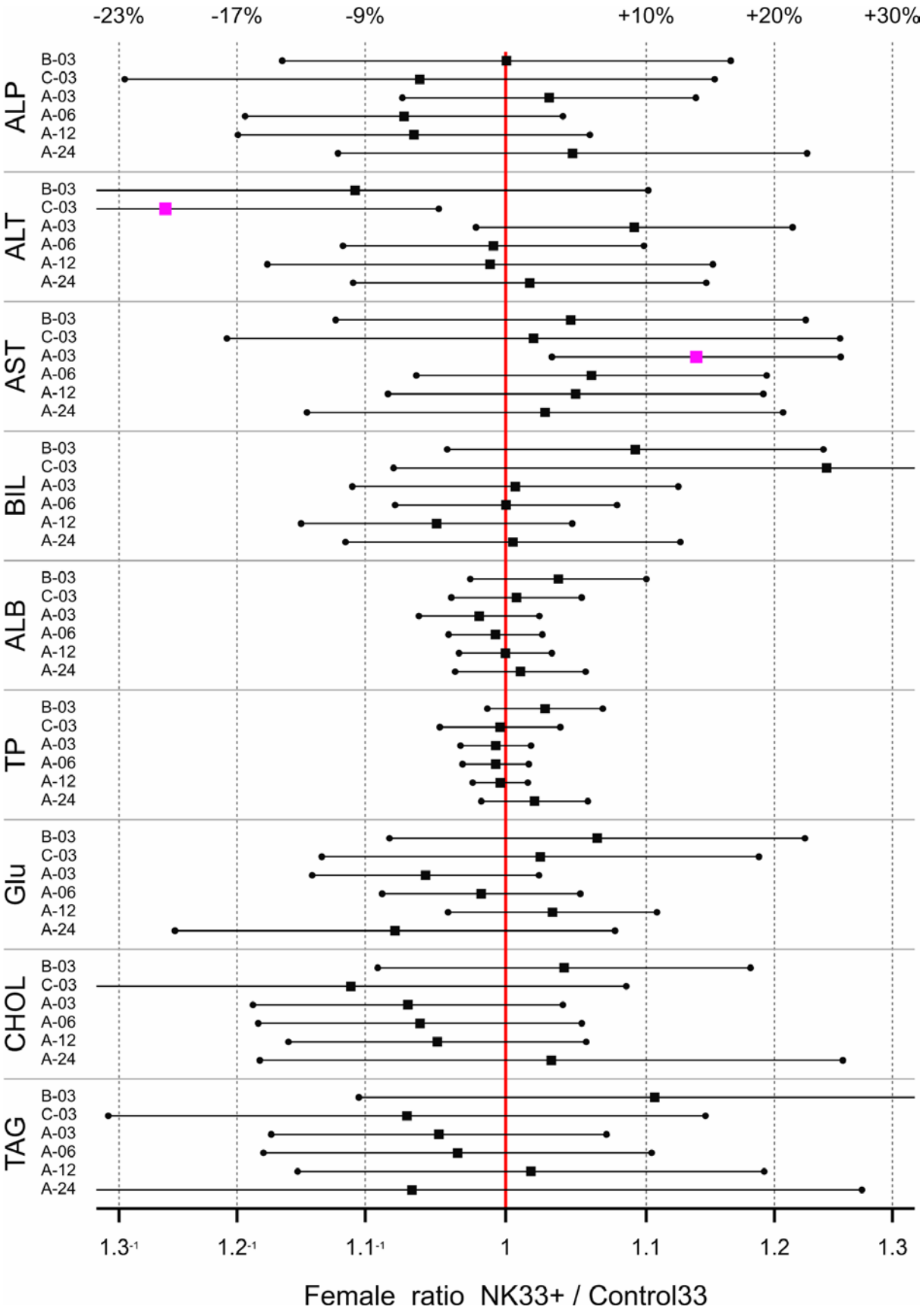


Figure 13 95% confidence intervals for Clin. Biochem. endpoints for NK33+ / Control for females.

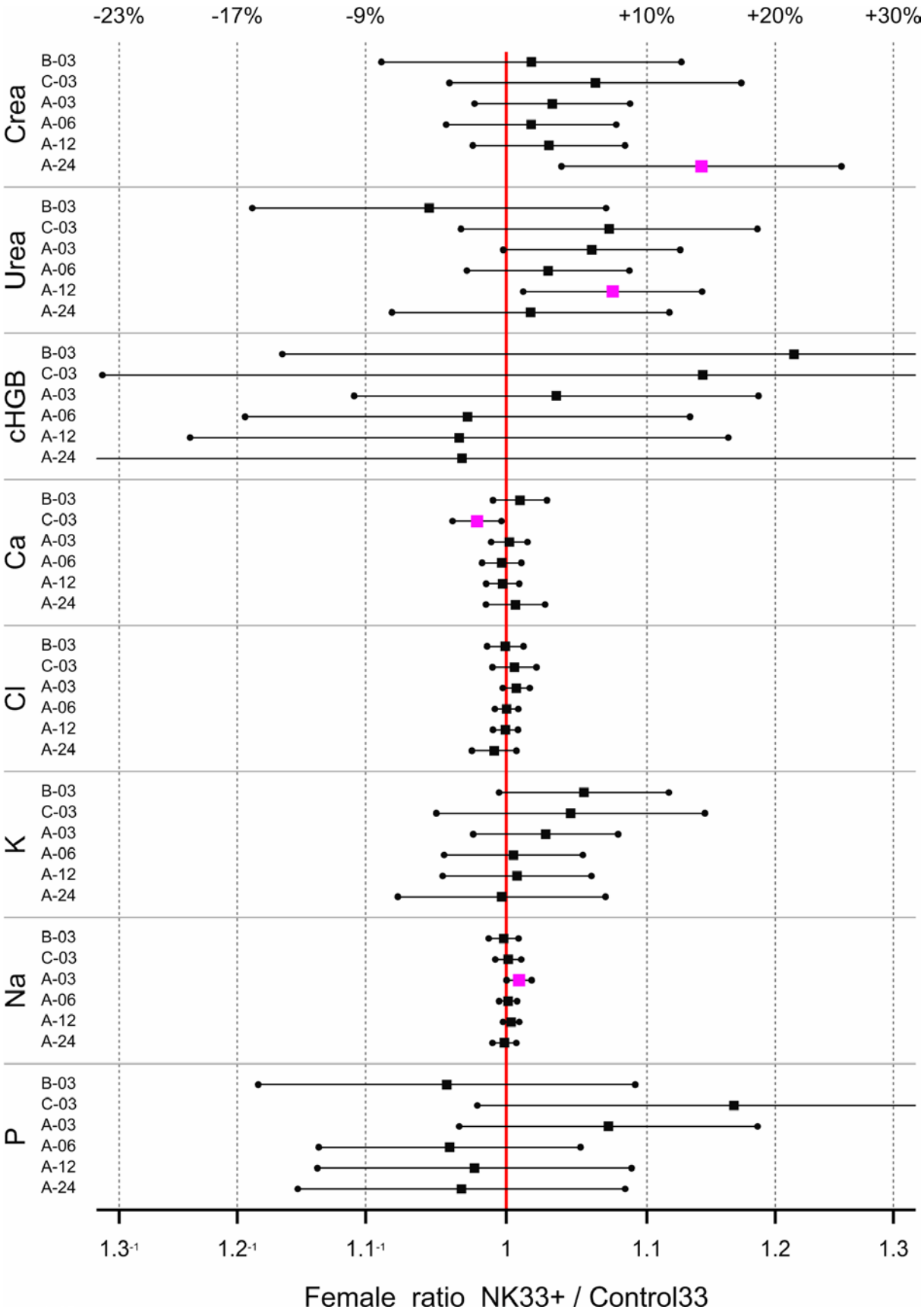


Figure 14 95% confidence intervals for Clin. Biochem. endpoints for NK33+ / Control for females.

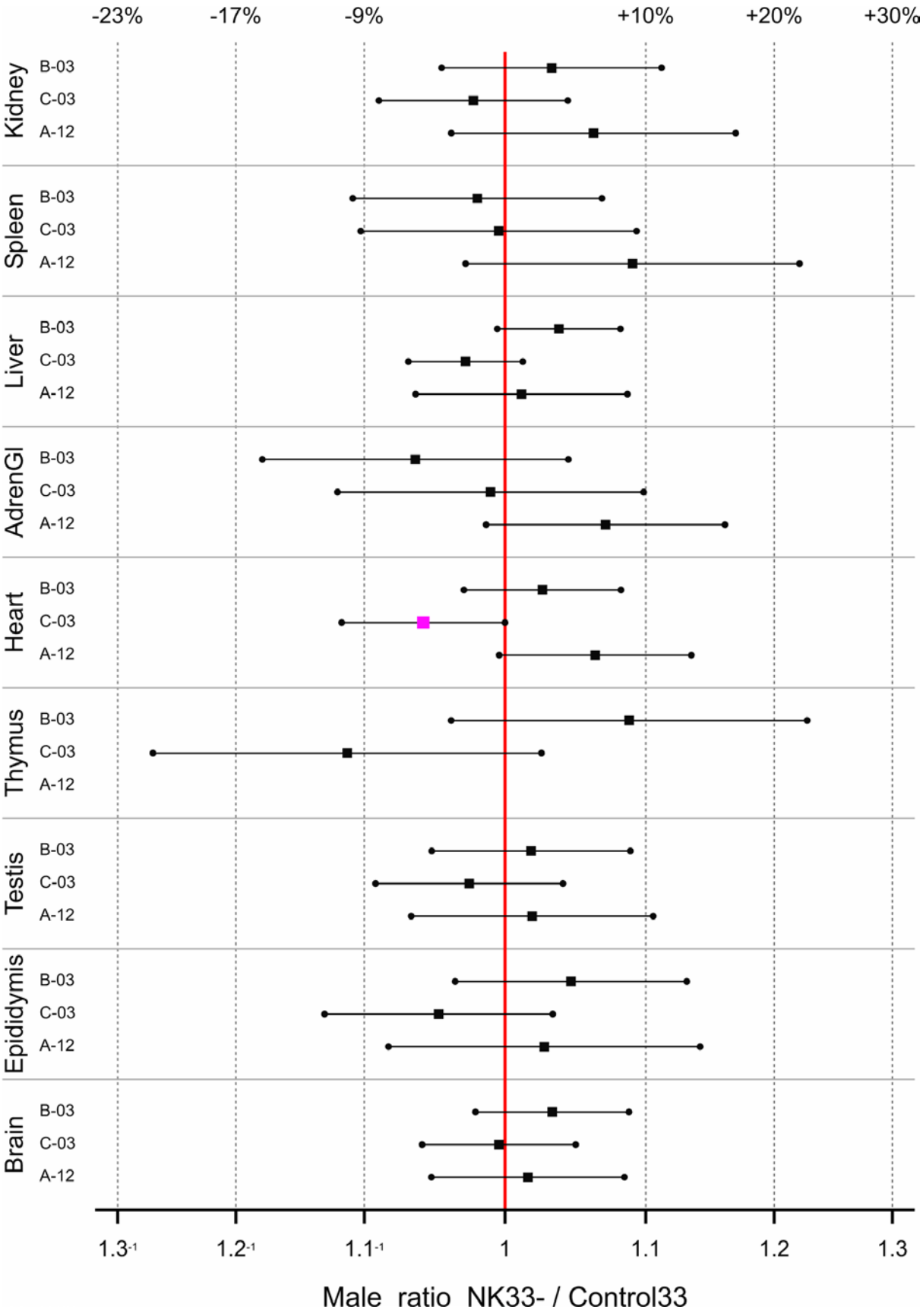


Figure 15 95% confidence intervals for Organ endpoints for NK33- / Control for males.

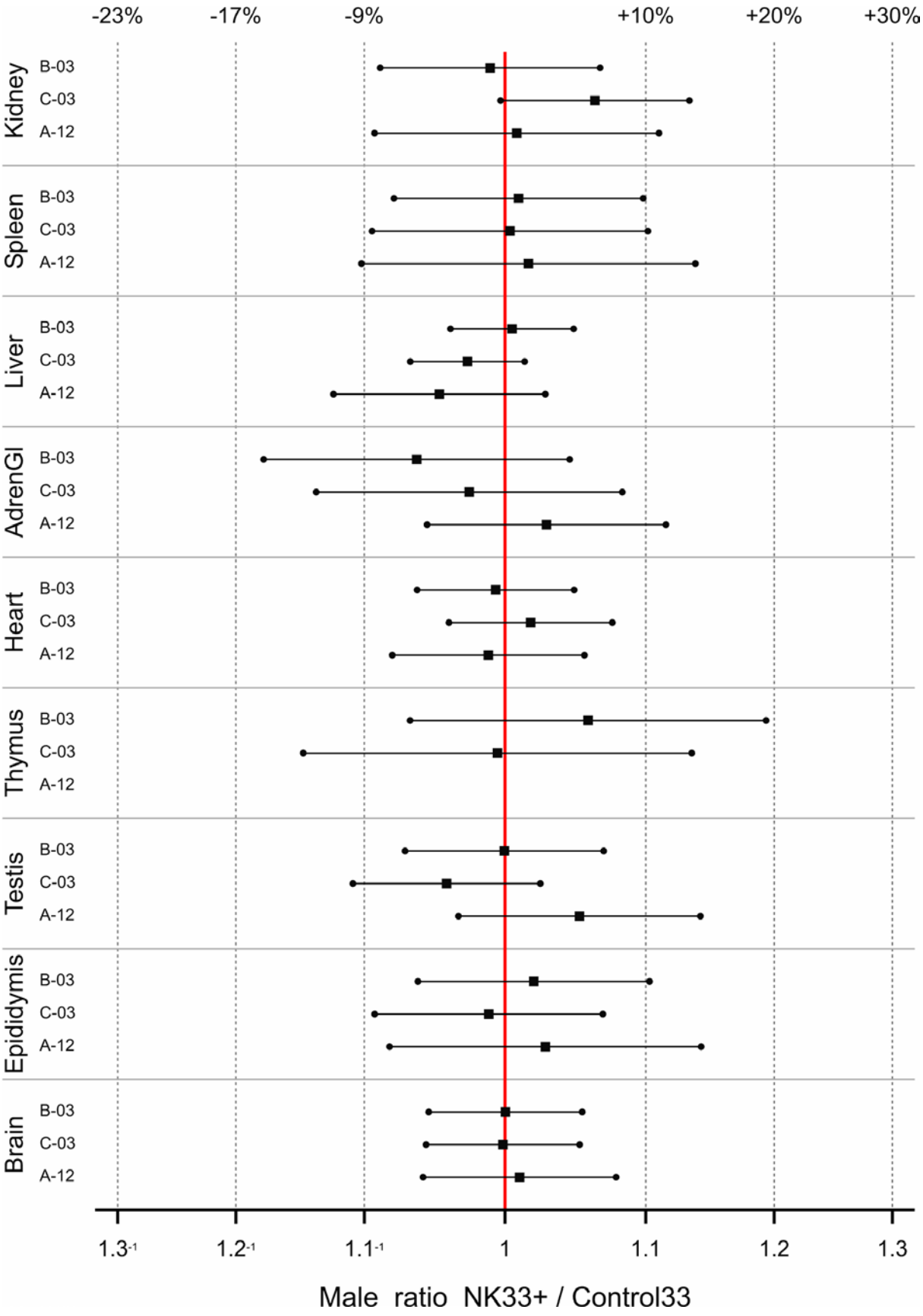


Figure 16 95% confidence intervals for Organ endpoints for NK33+ / Control for males.

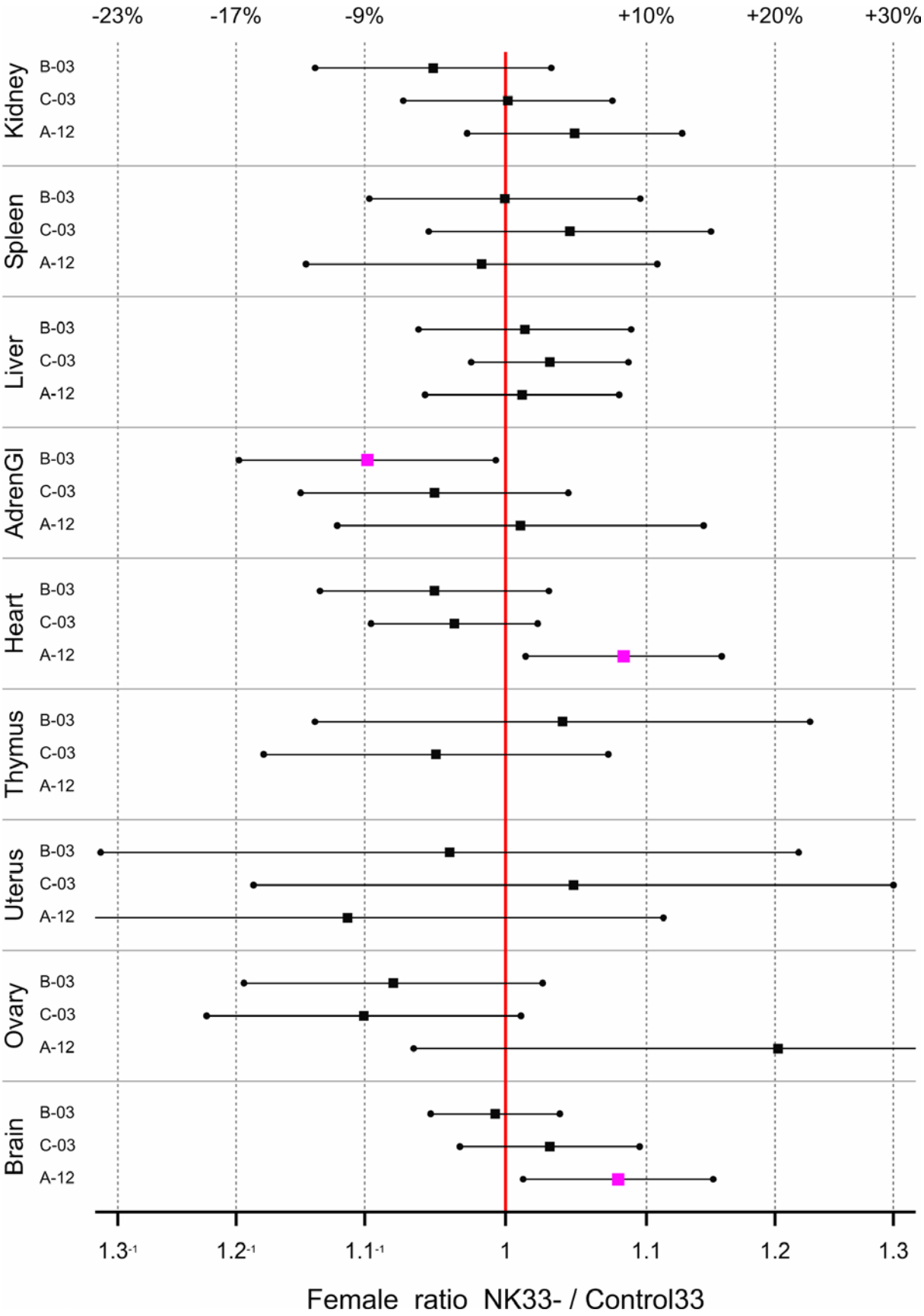


Figure 17 95% confidence intervals for Organ endpoints for NK33- / Control for females.

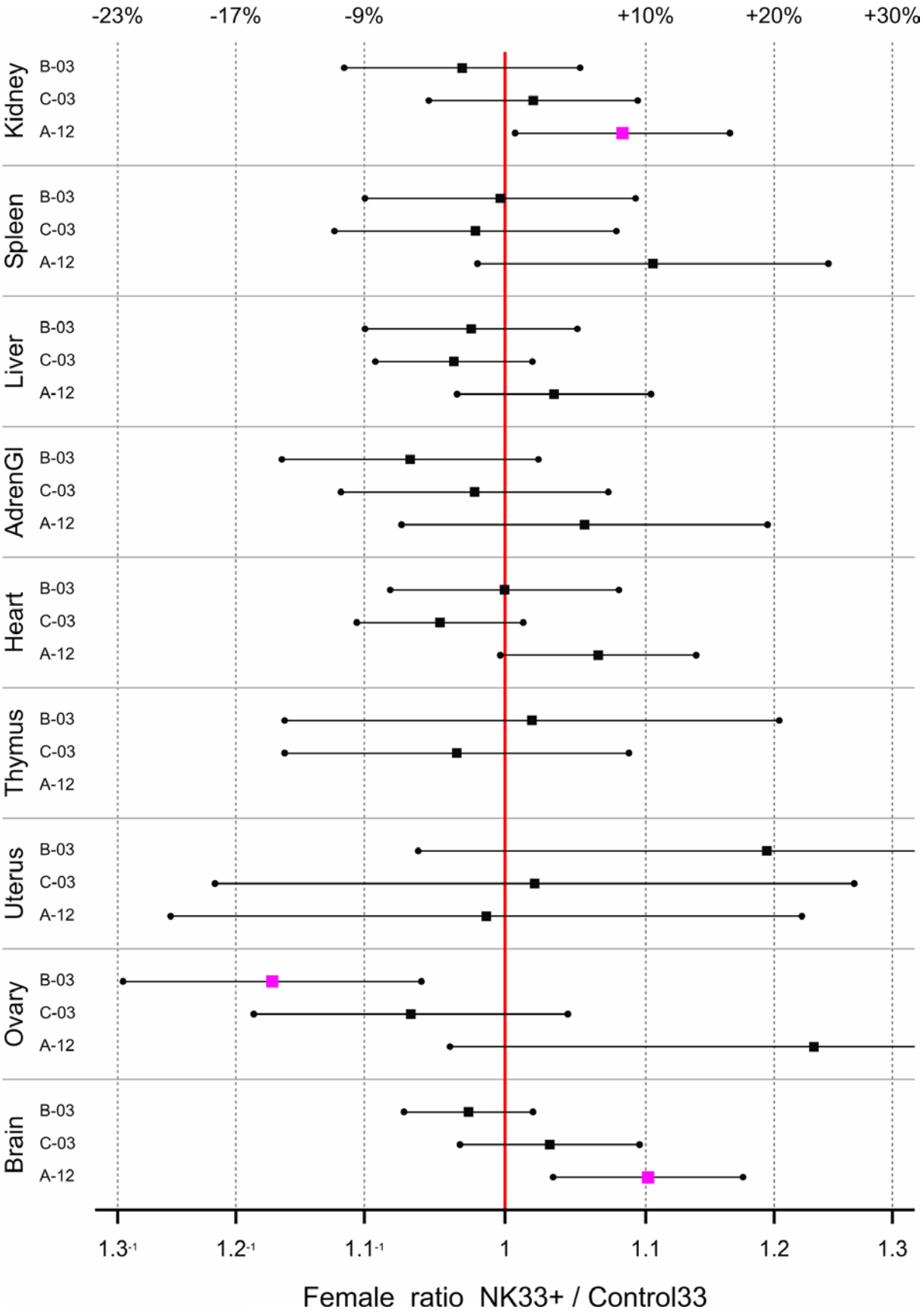


Figure 18 95% confidence intervals for Organ endpoints for NK33+ / Control for females.

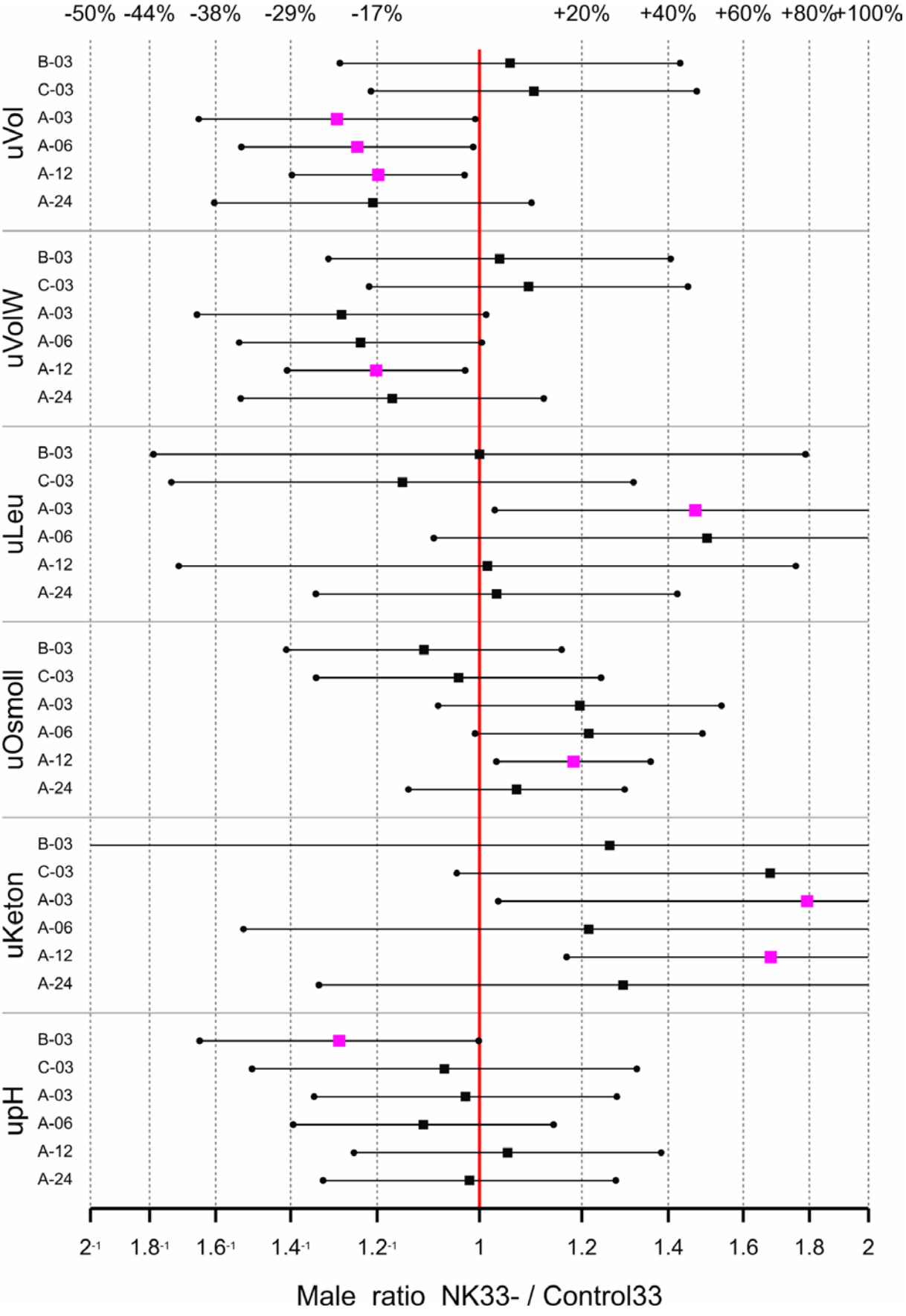


Figure 19 95% confidence intervals for Urine endpoints for NK33- / Control for males.

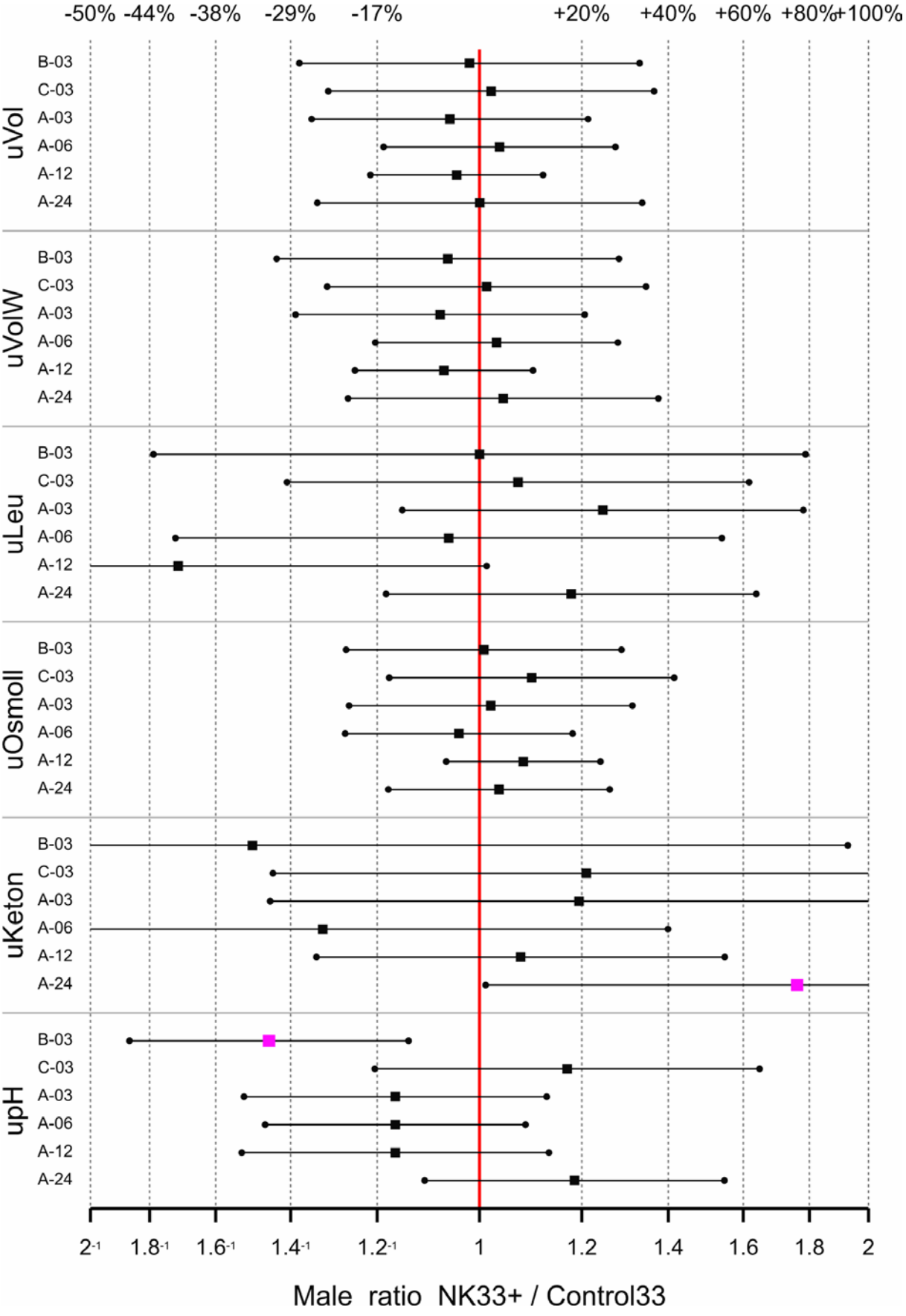


Figure 20 95% confidence intervals for Urine endpoints for NK33+ / Control for males.

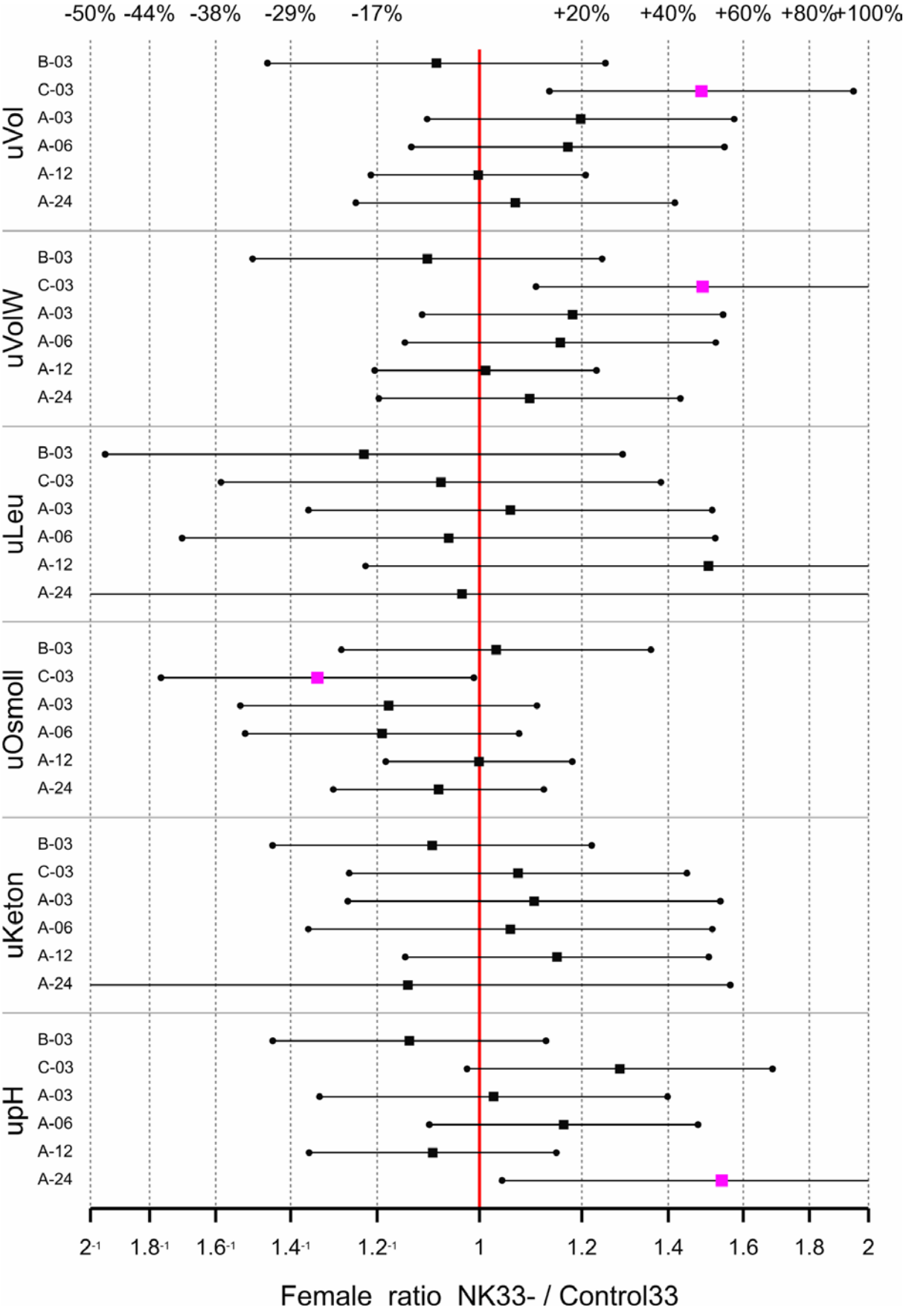


Figure 21 95% confidence intervals for Urine endpoints for NK33- / Control for females.

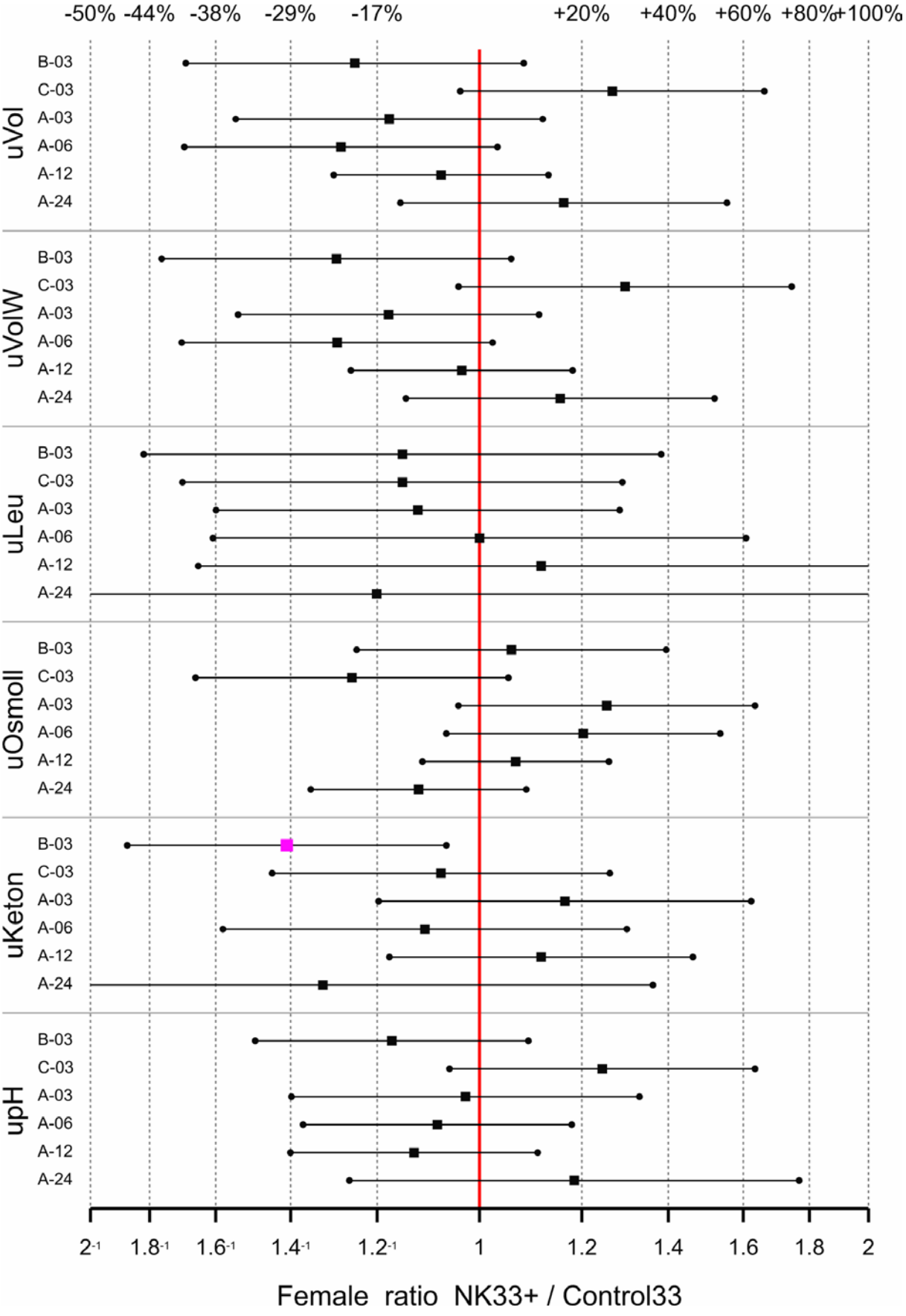


Figure 22 95% confidence intervals for Urine endpoints for NK33+ / Control for females.

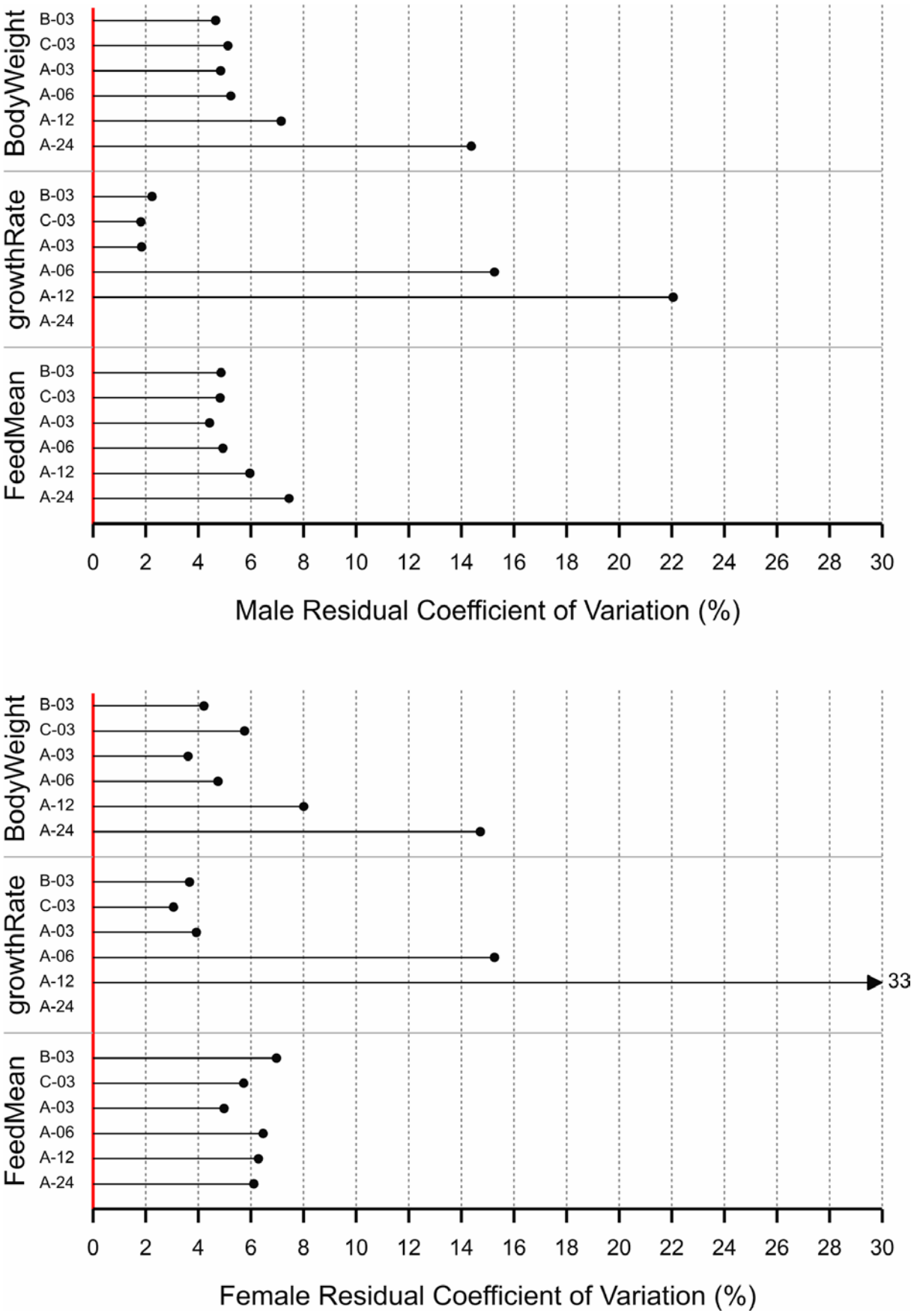


Figure 23 Coefficient of variation for Weights endpoints for males (top) and females (bottom).

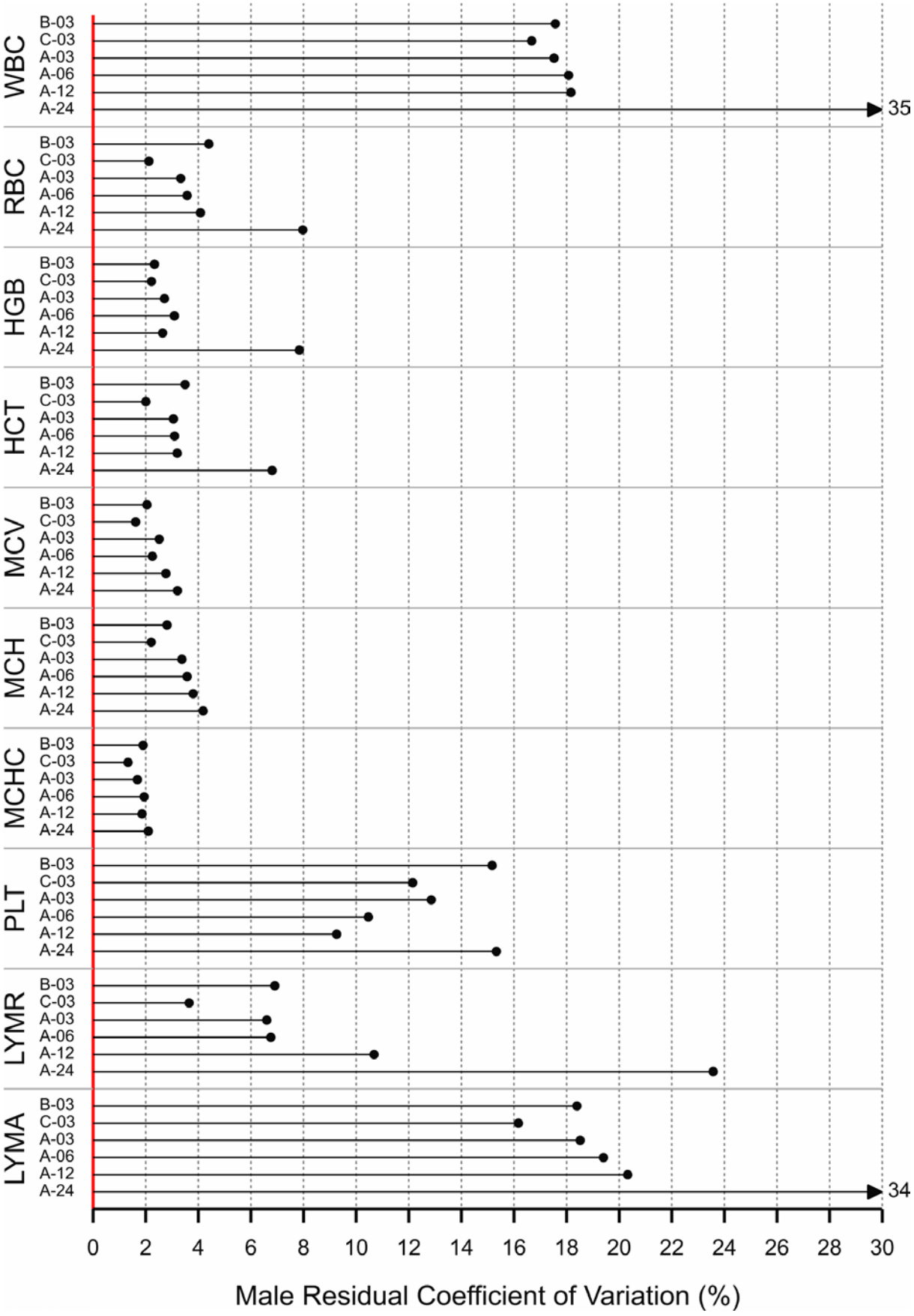


Figure 24 Coefficient of variation for Haematology endpoints for males.

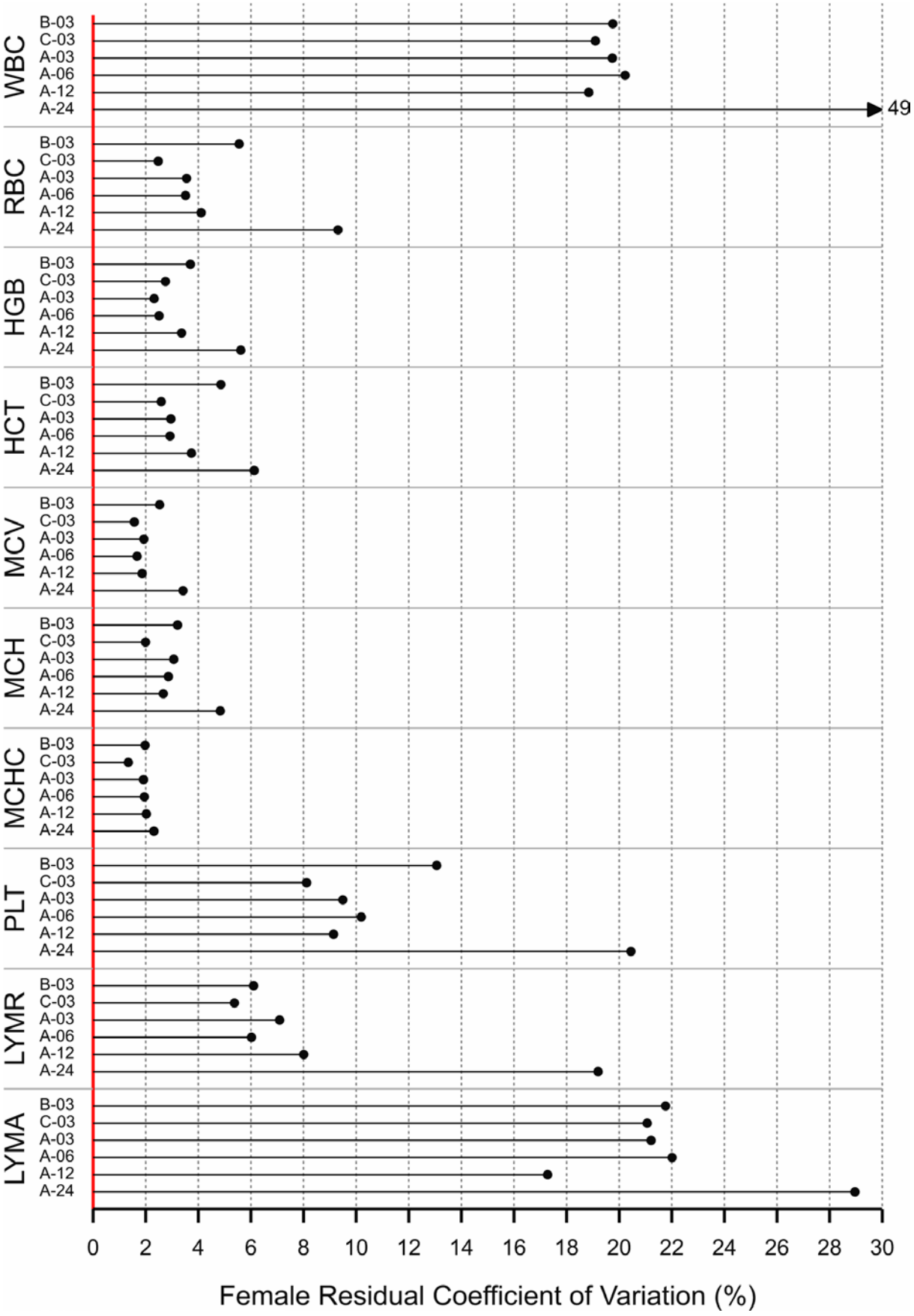


Figure 25 Coefficient of variation for Haematology endpoints for females.

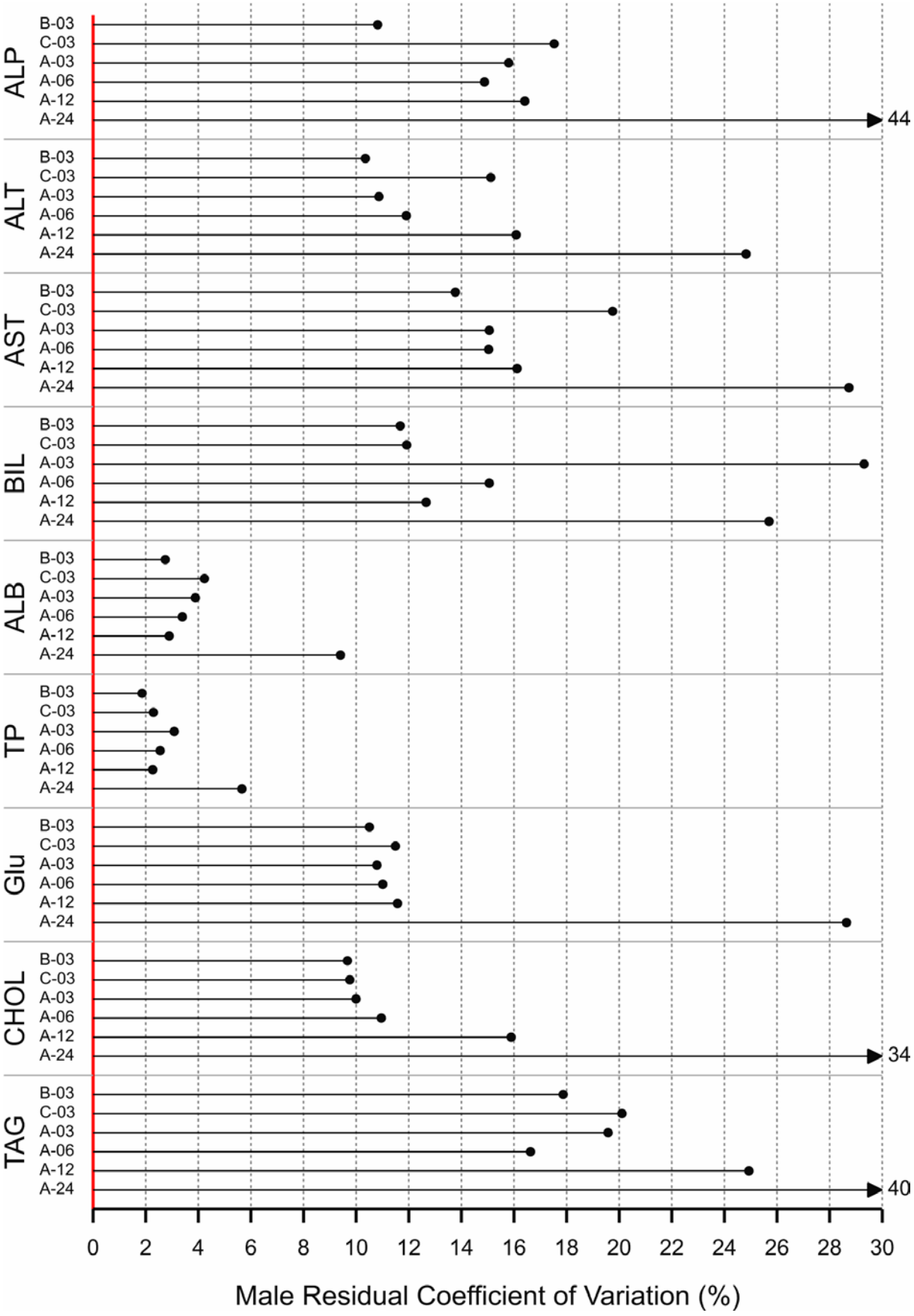


Figure 26 Coefficient of variation for Clinical Biochemistry endpoints for males.

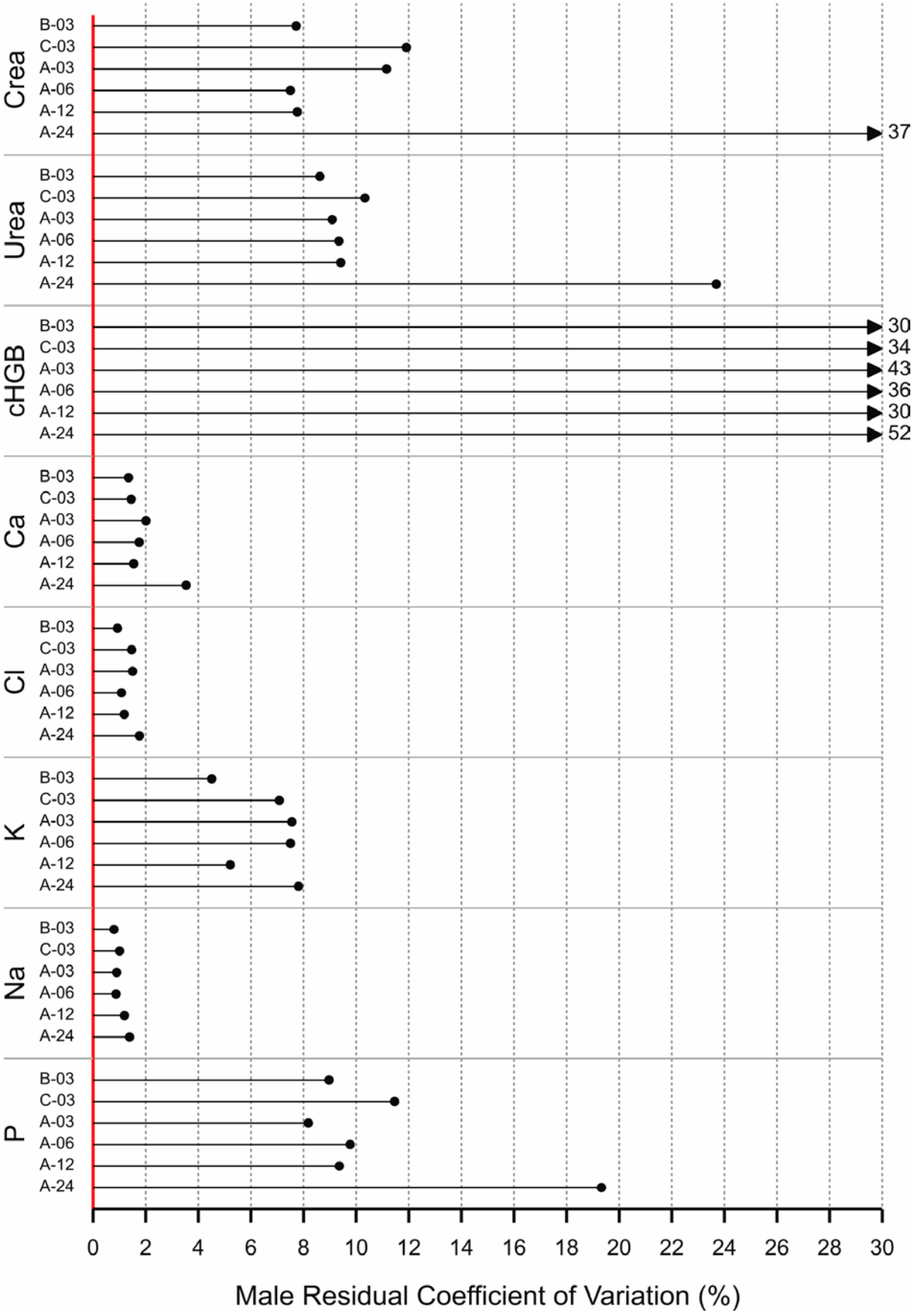


Figure 27 Coefficient of variation for Clinical Biochemistry endpoints for males.

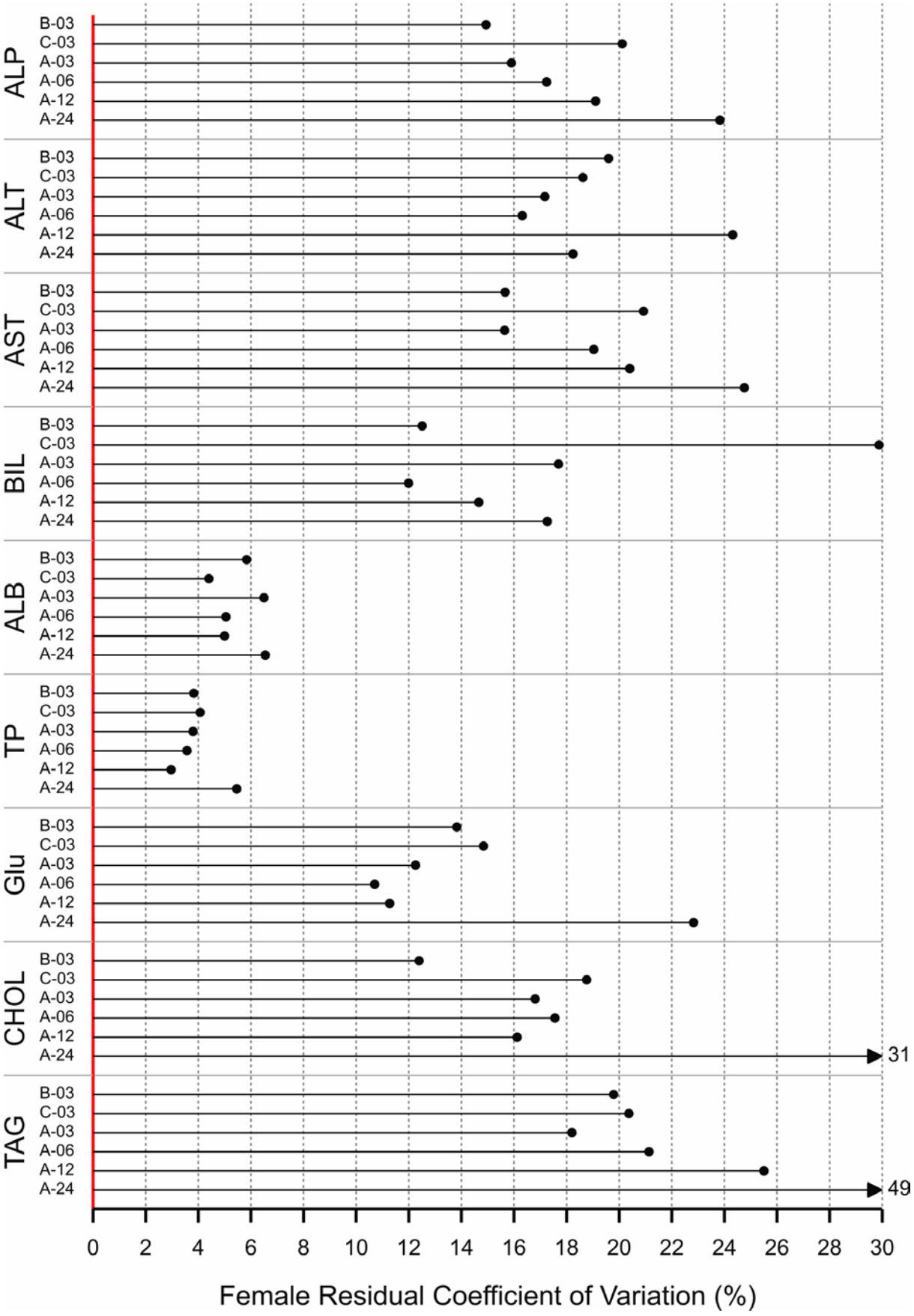


Figure 28 Coefficient of variation for Clinical Biochemistry endpoints for females.

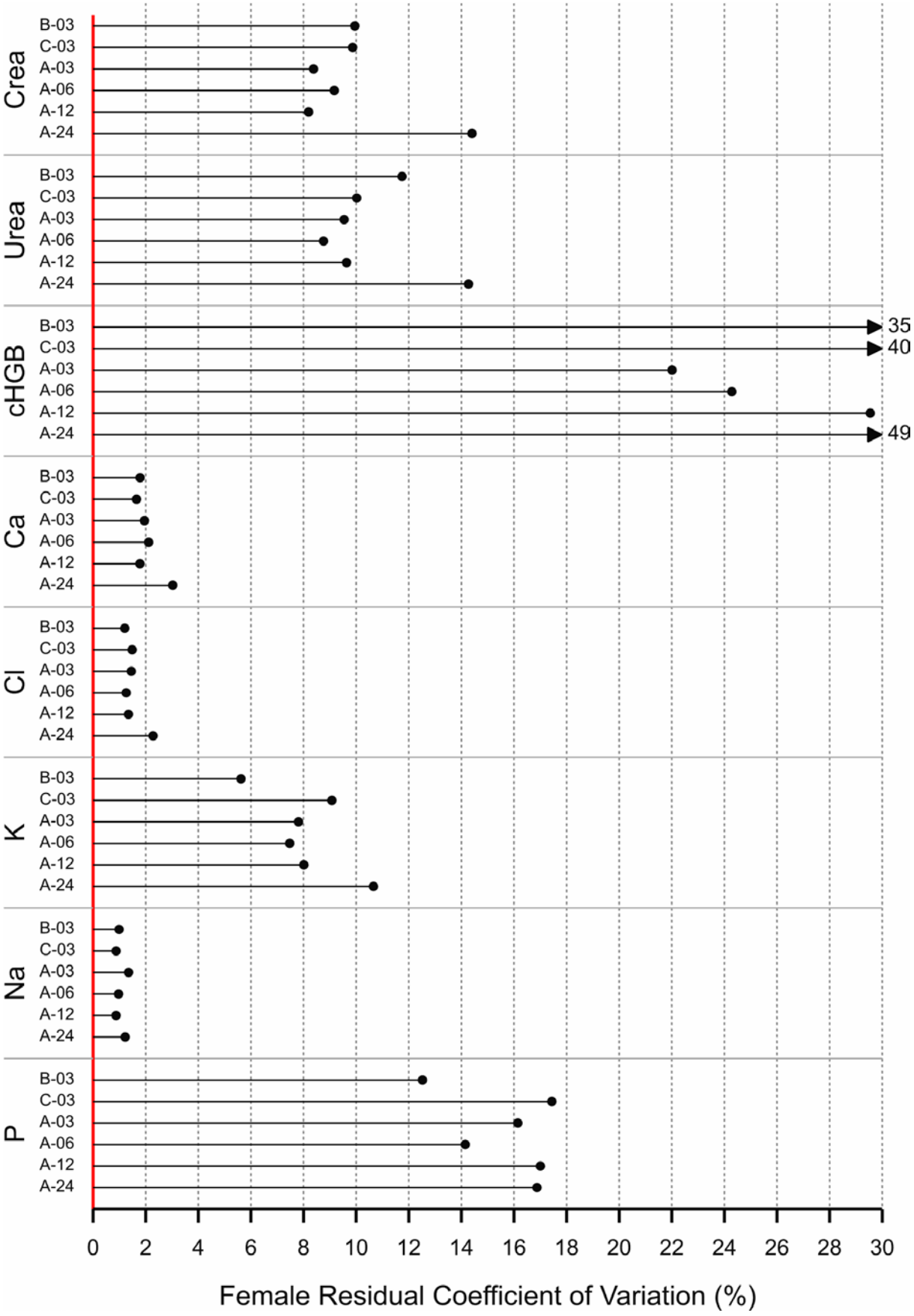


Figure 29 Coefficient of variation for Clinical Biochemistry endpoints for females.

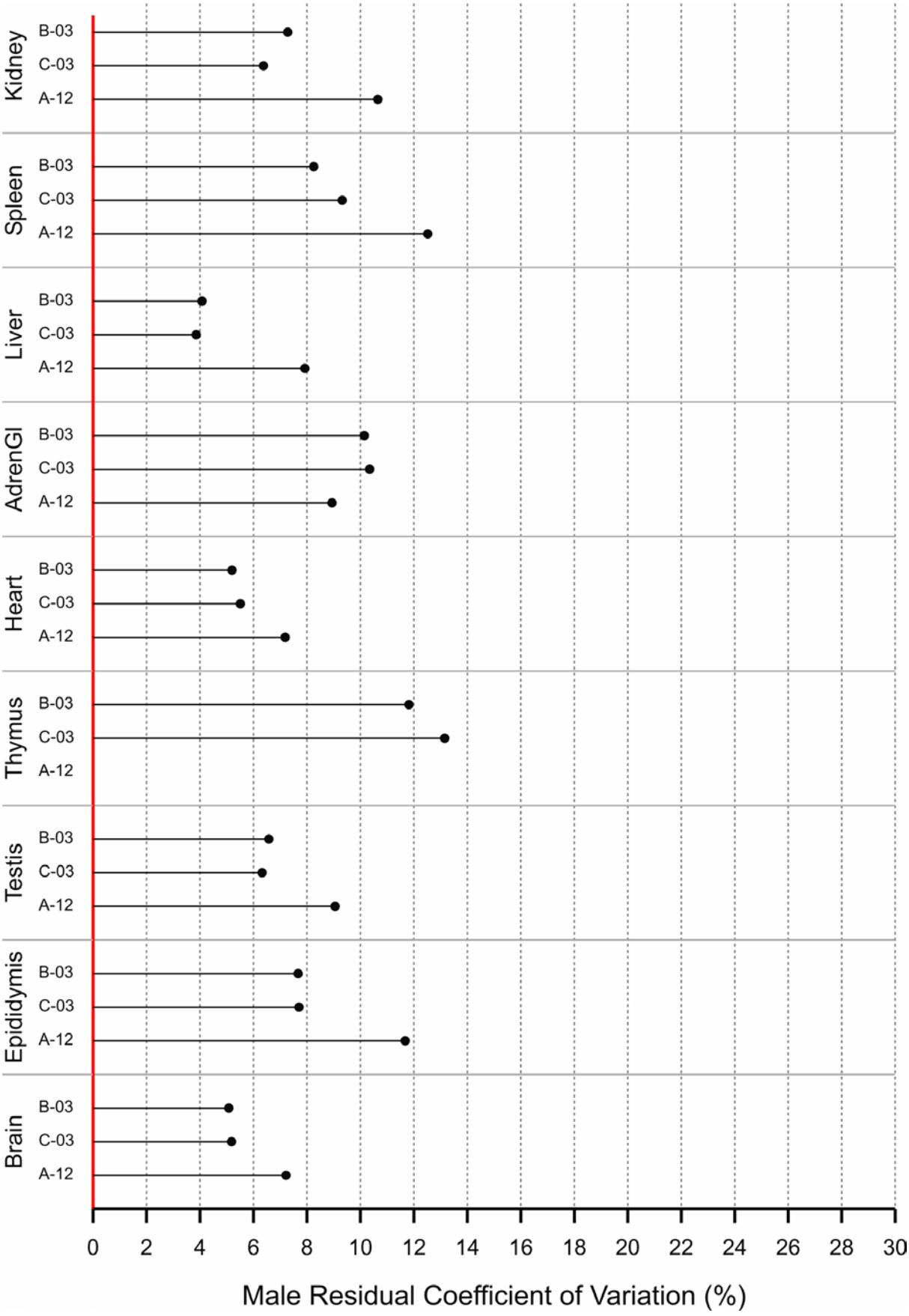


Figure 30 Coefficient of variation for Organ endpoints for males.

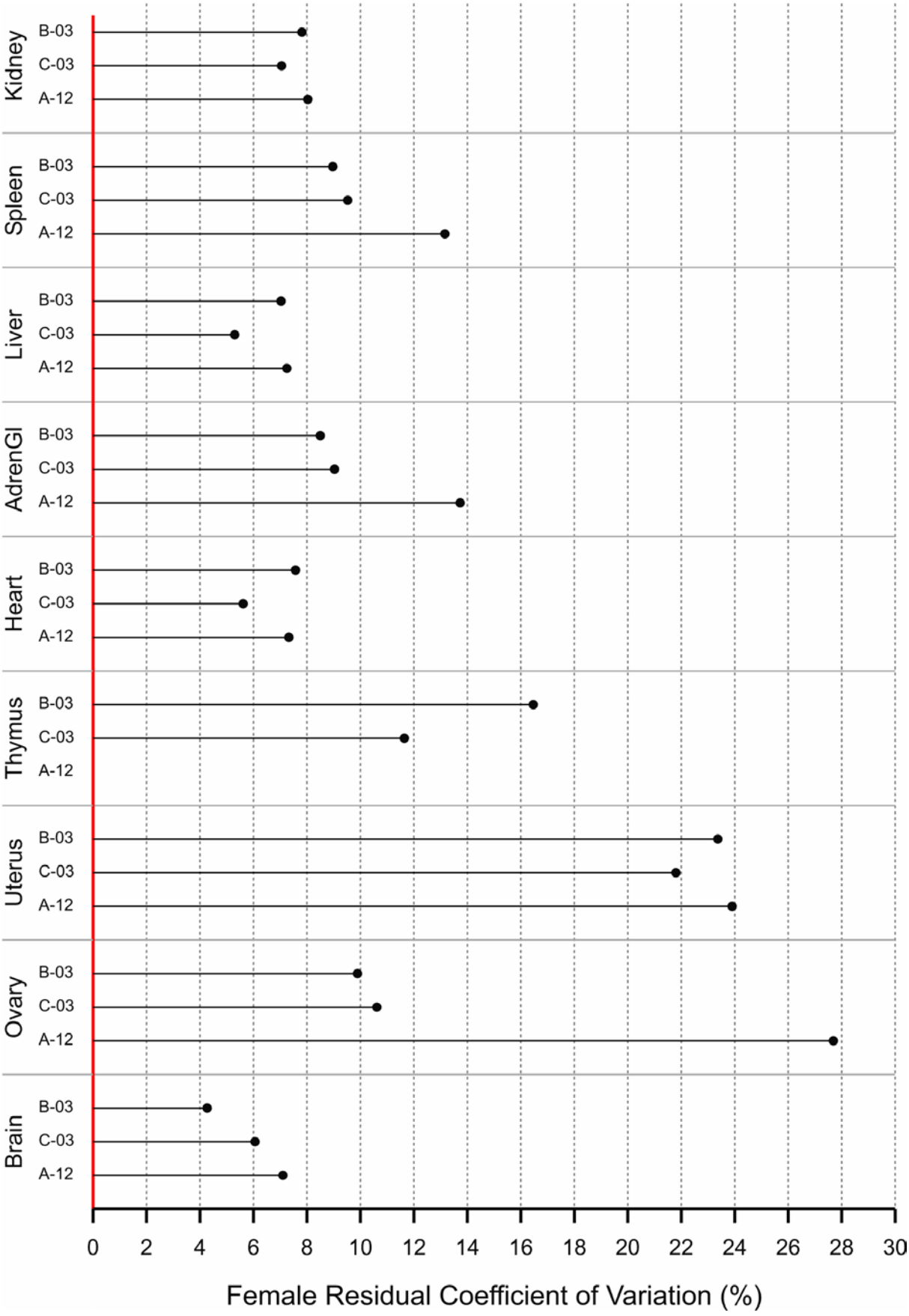


Figure 31 Coefficient of variation for Organ endpoints for females.

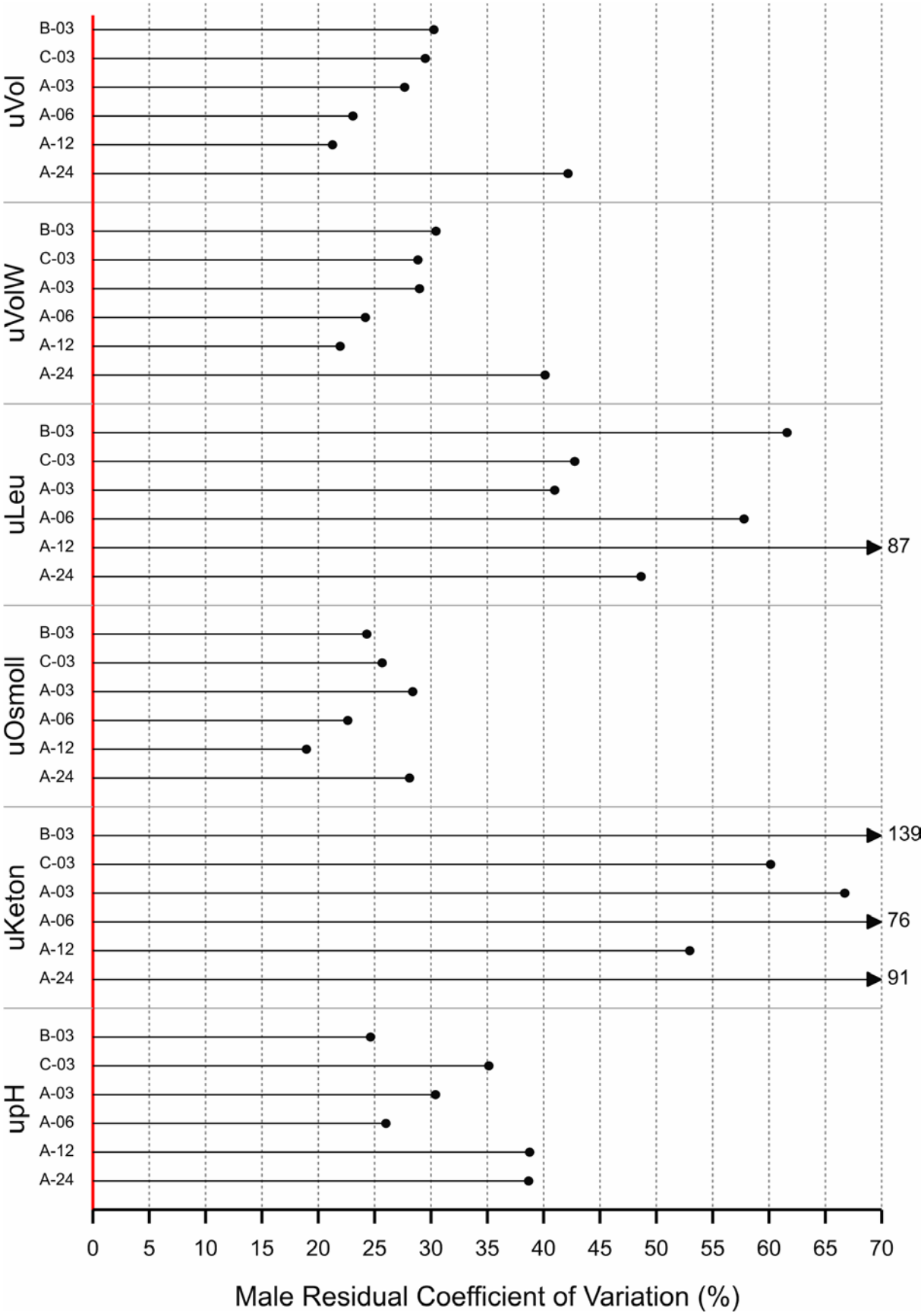


Figure 32 Coefficient of variation for Urine endpoints for males.

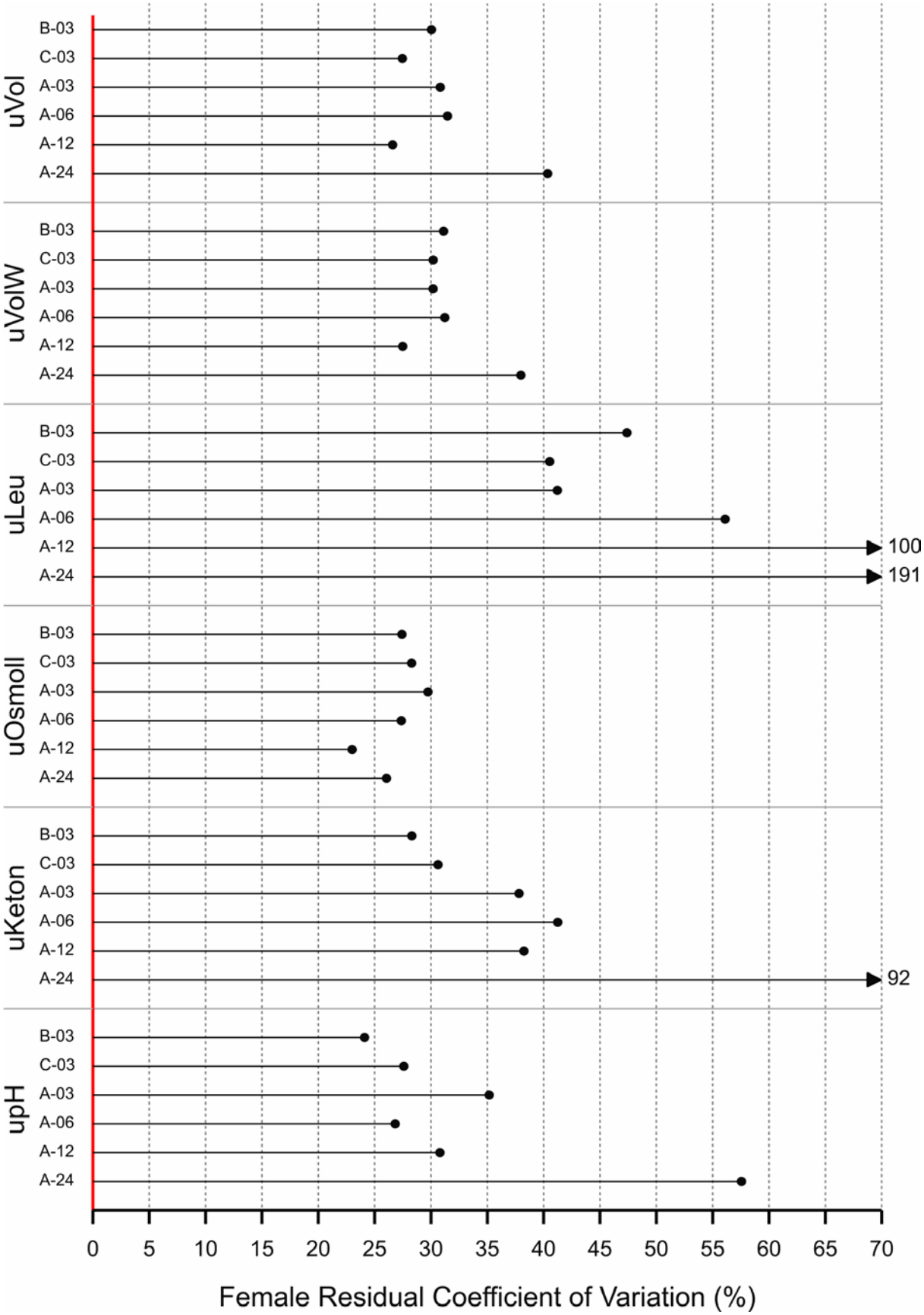


Figure 33 Coefficient of variation for Urine endpoints for females.