Objective
This study aimed to report the screening performance of cell free (cf)DNA testing for chromosomal abnormalities in multiple pregnancies.

Methods
Data were obtained from consecutive pregnant women with a multiple pregnancy or a vanishing twin pregnancy at >10 weeks’ gestation who requested for self-financed cfDNA testing between May 2015 and November 2019. Those that had positive screening results had diagnostic confirmatory procedures after detailed counselling and consent given. The performance of screening of the cfDNA test was determined by calculating concordance rate, confirmation rate and combined false-positive rate (cFPR).

Results
Data from 324 women were included. Median (interquartile range) maternal age was 34 (32–37) years. There were 29 cases with triplets, 276 cases with twins and 19 cases with vanishing twins. There were 15 (4.6%) cases with no result from the first blood draw, with 13 cases (86.7%) because of low fetal fraction; following subsequent blood draw 12 cases obtained results. For triplets, 20/29 cases (69.0%) had fetal reduction, either spontaneously (n=8) or iatrogenically (n=12); 10 cases had cfDNA testing done before the reduction and 10 cases were performed after. For twins, 30/276 cases (10.9%) had fetal reduction, either spontaneously (n=23) or iatrogenically (n=7); with 11 cases and 19 cases having cfDNA testing done before and after the reduction, respectively. The performance of fetal reduction in twins and triplets did not affect screening performance of cfDNA. Both concordance rates and confirmation rates for trisomy 21 (n=2/2) and 18 (n=1/1) were 100% and 100%, respectively, for twins. For sex chromosome aneuploidy (SCA), one case had high-risk result for any SCA with a concordance rate of 0% (n=0/1), with a cFPR of 12.5% (n=1/8) in triplets; 4 cases had high-risk result for any SCA with a concordance rate of 0% (n=0/4) in twins, with a cFPR of 2.6% (n=4/155). Amongst the cases with vanishing twin, the median interval between timing of demise to cfDNA testing was 6 (range 1 – 13) weeks. Two cases tested high-risk for trisomy 21; both cases had amniocentesis performed of the surviving twin showing 46, XX. Two cases tested high-risk for SCA and both cases resulted in a normal live birth; for one case the abnormal cfDNA result was considered to have originated from the vanishing twin as repeated cfDNA testing showed a reduction in fetal fraction. The other case declined further repeated testing, with ultrasound showing sex discordance (female) with the cfDNA test (low level of Y chromosome). The cFPR for cfDNA testing in vanishing twins was 23.5% (n=4/17).

Conclusion
Amongst the twin and triplet pregnancies, confirmation rate for trisomy 21 and 18 is 100%, at a cFPR of 12.5% for triplets and 2.6% for twins. Amongst the pregnancies with vanishing twins, the cFPR is up to 24%, which results in a high invasive testing rate or repeated cfDNA testing rate.