Introduction
Homologous recombination deficiency (HRD) assays determine eligibility for treatment with PARP inhibitors and may have use for other DNA repair targeting drugs. The assays measure several factors to define homologous recombination (HR) status including causes (i.e., inactivation in HR repair (HRR) pathway genes) and consequences (i.e., genomic scoring of HRD). Variability in determining HR status across assays has not been investigated thoroughly, and an empirical assessment of assay variability may support broader adoption of HRD and strengthen clinical interpretation of test results.

Materials & Methods
HRD Assays
Commercial and academic HRD assay developers were invited to participate in the project, resulting in 16 collaborations representing 15 different HRD assays. Factors measured to determine HR status (i.e., gLOH inclusion, TAI inclusion, LST inclusion, mutations in either BRCA1/2 HRR pathway genes) were provided by the test developers. For each sample, developers provided HRD status, score, and BRCA1/2 status. There are research use only (RUO) assays and laboratory-developed tests (LDTs) included in the analyses.

In Silico Samples
A subset of assay developers (n=11) received de-identified segmented files, MAF files, and BRCA1/2 germline mutation files for 348 TCGA ovarian cancer samples. Assay developers ran TCGA samples through their modified HRD pipeline to measure and report HR status and the contributing factor(s) for each sample. BRCA1/2 mutated samples were defined as samples included in the germline mutation file and samples which any group identified a somatic BRCA1 or BRCA2 alteration (n=83).

Patient Samples
Archival specimens (n=142) from patients with stage II–IV high grade serous ovarian cancer diagnosed between 2011 and 2022 were identified in a biorepository at the University of Alabama at Birmingham (UAB). UAB sectioned FFPE tumor from debulking surgery for the 99 samples with adequate tissue and Molecular Characterization Laboratory (MoCha) at the NCI Frederick National Laboratory performed DNA and RNA extraction. MoCha shipped identical aliquots of DNA and/or RNA from the 90 samples that possessed QC for independent sequencing and HRD measurement by 13 assays. BRCA1 and BRCA2 alterations were defined as clinical data from UAB, which included germline and somatic alterations.

Statistical Analysis
Statistics from the NCI Biometric Research Program performed pairwise comparisons of assays’ HR status calls to determine the level of agreement and considered specific factors measured by each assay to identify potential sources of variance for each dataset (in Silico Samples and Patient Samples were analyzed separately). Additionally, they analyzed HR status agreement for BRCA1/2 mutated versus wild type BRCA1/2 samples.

Patient Sample Results
The range of percent HRD positivity is 23–74% with a median of 53% and a mean of 54%. Assay developers (n=13) ran ovarian cancer patient samples (n=90) through their HRD pipelines and reported whether each sample was HRD or not. The percent of samples that were HRD out of all the samples was reported as the percent HRD for each assay. The assays are ordered by percent HRD here and throughout the analysis.

Agreement Among Samples with WT BRCA1 and BRCA2
PPA, APA, NPA, and ANA were computed for all possible pairings of samples (n=22) across all assays (n=13). Sample results were seen for in Silico Samples (data not shown).

Conclusions
This unique partnership allowed us to further understand similarities and differences among HRD assays. The median HRD positivity rate of 49% in the in Silico Analysis and 52% in the Patient Sample Analysis is consistent with prior publications.

Next Steps
• Perform additional analyses that examine the impact of clinical factors (e.g., platinum status, race), sample factors (e.g., DNA quality, tumor content, tissue origins in HRR pathway genes (e.g., RAD51C, PALB2) on HRD call concordance.
• PRIORI FRIENDS will host a public meeting on February 1, 2024.