# Supporting Information

# Immuno-hybridization chain reaction enhances detection of individual cytokine-secreting human peripheral mononuclear cells

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**Table S-1.** Four initiator oligomers (A1-A4) and four pairs of complementary hairpin oligomers (H1/H2 - H7/H8).

| | Name | Sequence |
|---|---|---|
| Initiator | A1 | 5' GCA CGT CCA CGG TGT CGC TTG AAT AAAAAAAAA |
| Hairpins | H1 | 5' ATT CAA GCG ACA CCG TGG ACG TGC ACC CAC GCA CGT CCA CGG TGT CGC ACC |
| | H2 | 5' GTT GCA CGT CCA CGG TGT CGC TTG AAT GCG ACA CCG TGG ACG TGC GTG GGT |
| Initiator | A2 | 5' GCA GCC GTA GAC TAG TGC GCG AAT AAAAAAAAA |
| Hairpins | H3 | 5' ATT CGC GCA CTA GTC TAC GGC TGC ACG ACC GCA GCC GTA GAC TAG TGC CAC |
| | H4 | 5' GTT GCA GCC GTA GAC TAG TGC GCG AAT GCA CTA GTC TAC GGC TGC GGT CGT |
| Initiator | A3 | 5' CGT CGG CAT CTG ATC ACG CGC TTA AAAAAAAAA |
| Hairpins | H5 | 5' TAA GCG CGT GAT CAG ATG CCG ACG TGC TGG CGT CGG CAT CTG ATC ACG GTG |
| | H6 | 5' CAA CGT CGG CAT CTG ATC ACG CGC TTA CGT GAT CAG ATG CCG ACG CCA GCA |
| Initiator | A4 | 5' CGT GCA GGT GCC ACA GCG AAC TTA AAAAAAAAA |
| Hairpins | H7 | 5' TAA GTT CGC TGT GGC ACC TGC ACG TGG GTG CGT GCA GGT GCC ACA GCG CTG |
| | H8 | 5' CAA CGT GCA GGT GCC ACA GCG AAC TTA CGC TGT GGC ACC TGC ACG CAC CCA |

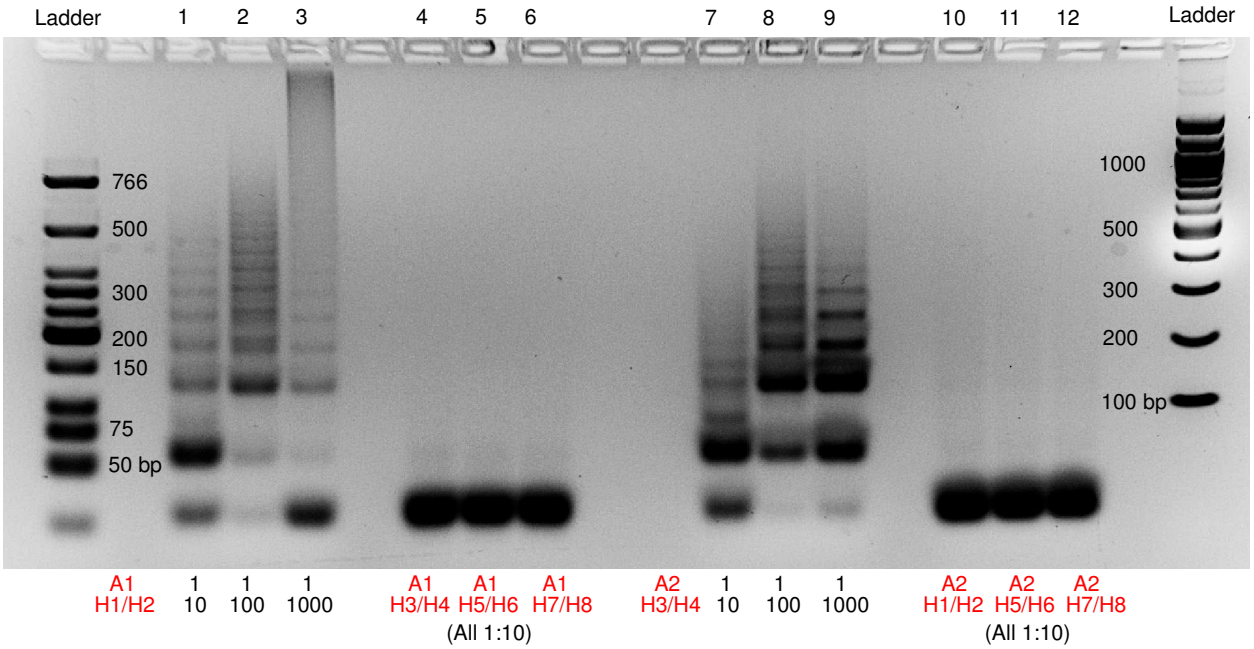


**Figure S-1. Characterization of the degree of polymerization by HCR and specificity by agarose gel electrophoresis**. Lanes 1-3: HCR extended bands from dilutions of initiator strand A1 to hairpins H1 and H2. Lanes 4-6: Initiator strand A1 mixed with not matching hairpin pairs at 1:10 molar ratio. Lanes 7-9: HCR extended bands from dilutions of initiator strand A2 to hairpins H3 and H4. Lanes 10-12: Initiator strand A2 mixed with not matching hairpin pairs at 1:10 molar ratio.

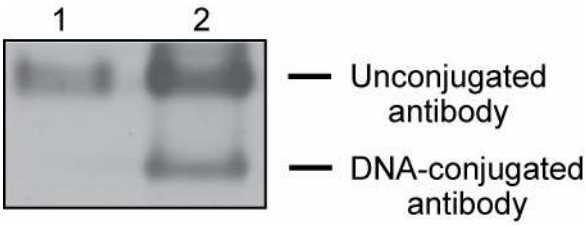


**Figure S-2. Analysis of initiator-antibody conjugation by native gel electrophoresis**. Lane 1: Anti-human IFNγ antibody (control). Lane 2: Antibody-oligonucleotide primer conjugation reaction with oligonucleotide initiator A1, sulfo-SMCC crosslinker, and anti-human IFNγ antibody.

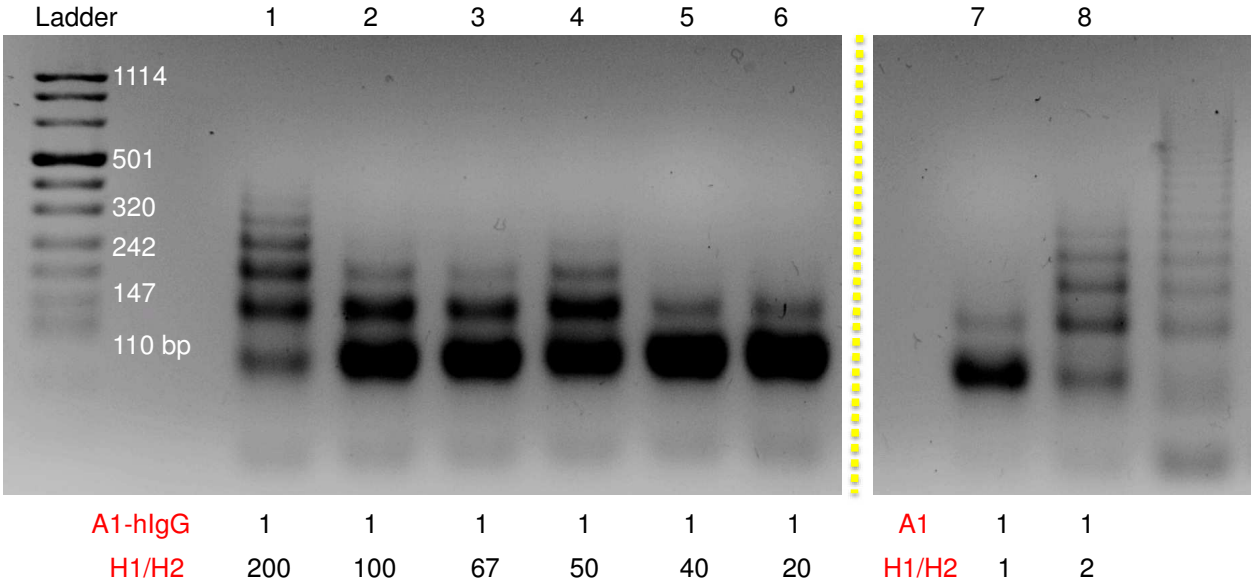


**Figure S-3. Analysis of HCR extension and estimation of the number of initiators on antibodies by gel electrophoresis**. Lanes 1-6: Initiator A1 conjugated anti human IgG antibodies and hairpins (H1/H2) were mixed at different molar ratios (1:200, 1:100, 1:67, 1:50, 1:40, 1:20). Lanes 7-9: Free initiator (A1) and hairpins (H1/H2) mixed at 1:1, 1:2 and 1:5 ratios. The unknown amount of initiator molecules on the antibody was estimated by running HCR with different dilutions of initiator A1 conjugated human IgG antibodies to hairpin H1/H2 mixtures (Lanes 1-6). HCR-extended chains on antibodies were compared to HCR-extended chains resulting from dilution series of free initiator and hairpin mixtures (Lanes 7-9) with known concentration of initiators. Based on the known amount of initiator and the extent of HCR, the ratio of initiator per antibody was calculated (1-2 initiator molecules per antibody).

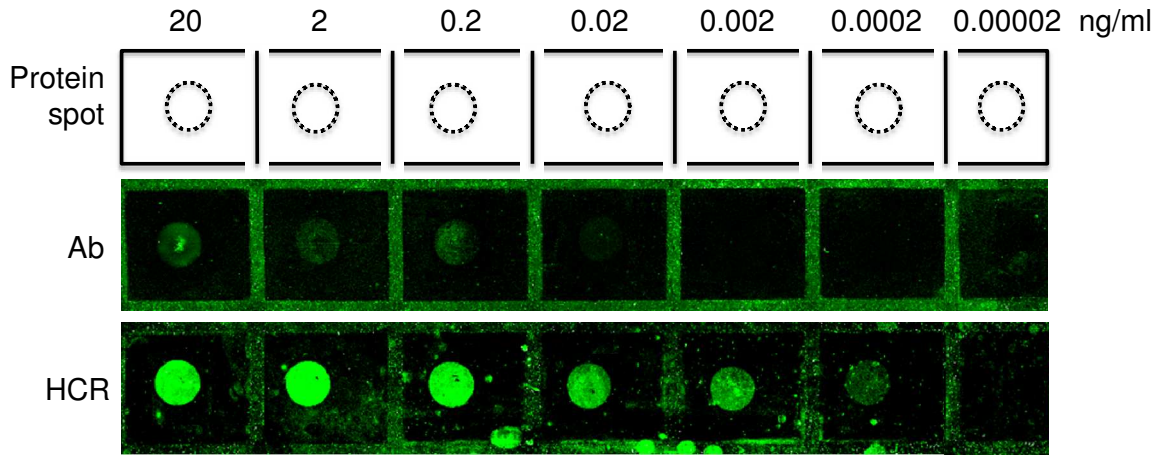


**Figure S-4. Representative micrographs of data comparing the detection of diluted cytokines by directly-labeled antibodies and immuno-HCR**. Anti-human IL-2 monoclonal antibody was spotted by hand on two identical poly-L-lysine-coated glass slides and human IL-2 cytokine was applied on both slides in a series of dilutions (20-0.00002 ng/mL). Captured IL-2 was detected either by a normal dye-conjugated secondary antibody detection or immuno-HCR method.

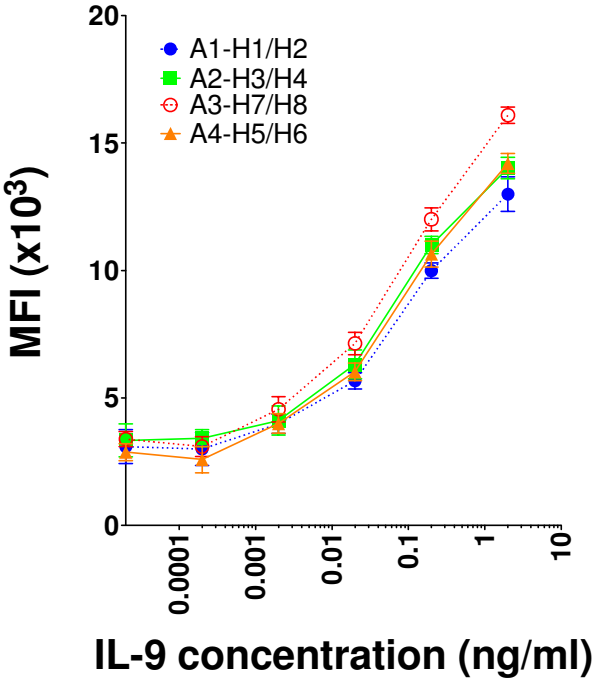


**Figure S-5. Titration curves generated for detection of IL-9 using each set of initiator and hairpins.** Monoclonal capture antibodies for human IL-9 were spotted on four different poly-L-lysine-coated glass slides and recombinant IL-9 cytokines were applied in a dilution series (2-0.00002 ng/mL) on each slide. Four preparations of detection antibodies for IL-9 conjugated with each initiator (A1-A4: Table S1) were incubated with each slide. Each hairpin pair (H1/H2, H3/H4, H5/H6, H7/H8: Table S1) was then loaded on each slide and the efficiencies of each immuno-HCR were compared.

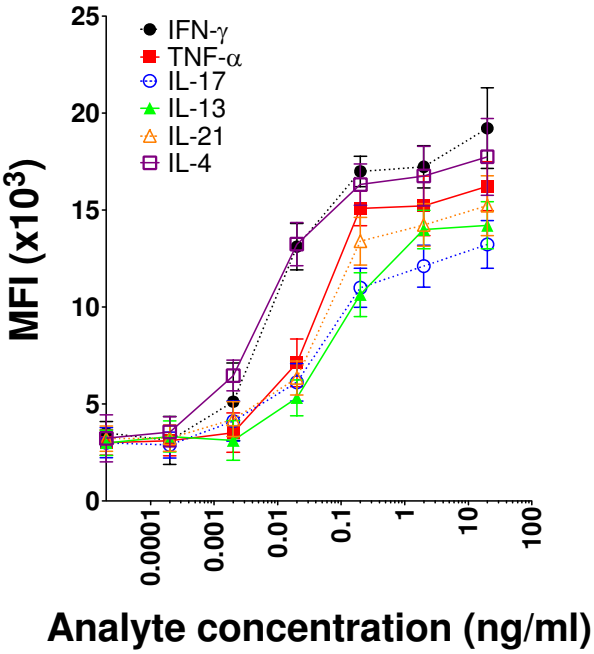


**Figure S-6. Titration curves for determination of the limit of detection for six human cytokines.** Monoclonal capture antibodies were spotted on poly-L-lysine-coated glass slides and cytokines were applied in a dilution series (20-0.00002 ng/mL). Spots were applied by hand on each slide and were detected by the immuno-HCR method.

**Table S-2. Limit of detection (LOD) and relative sensitivity of immuno-HCR method for the detection of 10 human cytokines.**

| | | Limit of Detection (LOD)[a] | | | Sensitivity[b] |
|---|---|---|---|---|---|
| **Cytokine** | **MW** | **HCR (pM)** | **Direct Ab (pM)** | **Fold-increase** | **Fold-Increase** |
| IL-2 | 15000 | 0.27 | 145.1 | 548 | 487 |
| IL-9 | 36000 | 0.96 | 4.0 | 4 | 4 |
| IL-10 | 20517 | 1.7 | 116.2 | 68 | 99 |
| MIP-1β | 7800 | 0.53 | 114.9 | 217 | 164 |
| IFNγ | 17100 | 0.56 | ND | ND | ND |
| TNFα | 51000 | 0.35 | ND | ND | ND |
| IL-17 | 15600 | 2.2 | ND | ND | ND |
| IL-13 | 15816 | 2.1 | ND | ND | ND |
| IL-21 | 15600 | 1.7 | ND | ND | ND |
| IL-4 | 17492 | 0.54 | ND | ND | ND |

a. Values were calculated from calibration curves where the LOD = Background (0 ng/mL analyte) + 3*σ/s (σ = the standard deviation of the lowest concentration of analyte measured in a given assay, s = slope of calibration curve in the linear range).

b. Relative increase in sensitivity was determined by the ratio of the slopes of the calibration curves in the linear range. ND means not determined.