

each of the antigen sites. The IgG print concentration were adjusted such that the positive control signals fell into an appropriate range.

**[0161]** Images from four clinical plasma samples are shown in FIG. 29, along with background-subtracted reaction site signal intensities for the pathogen-specific antigens. The clinical sample images in FIG. 29 are a representative sampling of images generated in the 28 minute cartridge assay. Positive and negative controls are present in these exemplary assays, and the printed antigen sites show different intensities for different individual samples. Based on reference test methods, samples (A) through (C) are each reactive for one pathogen and negative for the other two. Sample (D) is antibody reactive for all pathogens as indicated by all array antigen sites yielding positive signal. In particular, samples (A) to (C) are each mono-infected, with reactivity to HIV Ab (A), *T. pallidum* Ab (B), and HCV Ab (C). Sample (D) has Ab reactivity to all three pathogens both in the reference methods and the assay system, as described herein.

**[0162]** The fluorescence arrays are also quantitative. The table in FIG. 30 provides quantitative antibody-antigen signal intensities associated with each reaction site. Results for each reaction site in the array are reported as background-subtracted and normalized fluorescence signal intensity. Background signal is an average of the signal on negative reference sites adjacent to the antigen site of interest. The intensity of the printed human IgG site signal is utilized for normalization; and the reported intensity is thus a dimensionless number. Slightly negative numbers result when the signal on the antigen site is lower than the adjacent negative control. All negative numbers encountered are invariably close to zero. Dynamic range of the current reader instrument is approximately 3.5 logs. Normalization by the signal of the anti-human IgG spots compensates for potential differences in coupled and collected light between different instruments or cartridges, as well as for possible changes in the fluorescent conjugate solution. Decisions about the reactivity of a sample with each disease antigen are based on comparing the normalized antigen signal to a pre-determined cutoff value. For each antigen, the cutoff value is established by reviewing the signals obtained from a large number of assays with samples known to be positive and negative for each disease. The cutoff value may be chosen to accurately distinguish positive from negative signals for the specific antigen. Significant changes to the antigens and the print or assay conditions may require establishment of new cutoff values for the various antigens in the assay.

**[0163]** FIGS. 31 and 32 illustrate details of an exemplary software processing used to calculate the results as summarized in the table in FIG. 30. FIG. 31 shows a flow chart, illustrating a process 3100 for operating a reader instrument, in accordance with an embodiment. Process 3100 is initiated by a start step 3102, which may be actuated by, for example, the insertion of cartridge 3110 into reader instrument 100. A camera (or another suitable imaging device, e.g., sensor 128 of FIG. 2) is connected within reader instrument 100 and brought online in a step 3110. The camera is calibrated in a step 3112. The calibration of the camera may be performed by, for example, reading in externally-supplied calibration data and adjusting the camera settings as appropriate. In a decision 3120, a determination is made as to whether a sample is ready within reader instrument 100. If, for example, cartridge 110 is incorrectly inserted into reader instrument 100 such that cartridge 110 cannot be correctly imaged by the

camera, then the result of decision 3120 is "NO", the sample is not ready, and process 3100 proceeds to a step 3130 to exit the program, and the process is ended in an end step 3132.

**[0164]** Continuing to refer to FIG. 31, if the result of decision 3120 is "YES", the sample is ready within reader instrument 100, and process 3100 proceeds to a step 3142, in which a light source is enabled. The light source may be, for example, a laser or a light-emitting diode (LED) of an appropriate wavelength and output power for illumination of the sample being evaluated. Additionally, a "watchdog circuit" may be activated, so as to safely power down the light source in case of any malfunction. In a step 3144, the exposure time of the light source is set. For example, a certain number and durations of exposure times may be preset in process 3100. An image frame is acquired in a step 3146, and the acquired image is saved to memory in a step 3148. In a decision 3150, a determination is made as to whether images have been taken for all preset exposure times. If the result of decision 3150 is "NO", not all exposure times are done, then process 3100 returns to step 3144, and the exposure time setting and image acquisition steps are repeated. If the result of decision 3150 is "YES", images have been acquired at all preset exposure times, then the light source is disabled in a step 3152. The acquired and saved images are then analyzed in a step 3154, and the analysis results are saved to memory in a step 3156. As an alternative, step 3154 and 3156 may be performed at peripheral device 1980 of FIG. 19, such as an external computer, rather than being integrated onboard reader instrument 100. Following step 3156, process 3100 returns to decision 3120. If a new sample cartridge has been inserted, then steps 3142-3156 are repeated. Otherwise, process 3100 is ended with exit program step 3130 and end step 3132.

**[0165]** Referring now to FIG. 32, further details of step 3154 of FIG. 31 are described. Step 3154 is initiated at a start step 3202 then, in a step 3204, a user manually inputs a cartridge identification number ("cartridge ID") into reader instrument 100. Alternatively, reader instrument 100 may include a barcode reader arrangement. In this case, a barcode illumination is enabled in a step 3210, and a barcode, including cartridge ID and placed on cartridge 110, is read into reader instrument 100. Based on the cartridge ID, the appropriate cartridge configuration information is read into reader instrument 100. Cartridge configuration may include, for example, specific reagents used on cartridge 110, layout of any printed protein array sites, and any other factors that may influence the image analysis. Once the cartridge configuration information has been read in step 3220, then reaction sites are located in all saved images in the memory, in a step 3222. For example, for cartridges with circular printed antigen sites, step 3222 may involve circle finding routines. In a step 3224, the acquired images for each reaction site for the various exposure times are compared, and the "best" acquired image for each reaction site is identified. For instance, the selected reaction site may correspond to the longest exposure time that does not saturate the camera. Then, in a step 3226, a grid corresponding to the printed protein array (such as shown in FIGS. 22 and 23) is located. For instance, step 3226 may include identifying the centers of the circles found in step 3222, combining this data with geometry information included in the cartridge configuration, then refining the analysis according to a least squares fit of predefined parameters in the cartridge configuration.

**[0166]** Still referring to FIG. 32, a reaction site signal is calculated for each reaction site in the printed protein array in