European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:883–890
https://doi.org/10.1007/s10096-019-03487-1

ORIGINAL ARTICLE

Plasmonic gold chips for the diagnosis of *Toxoplasma gondii*, CMV, and rubella infections using saliva with serum detection precision

Xiaoyang Li[1] · Christelle Pomares[2] · François Peyron[3] · Cynthia J. Press[4] · Raymund Ramirez[4] · Gonfrier Geraldine[5] · Isabelle Cannavo[5] · Emmanuelle Chapey[6] · Pauline Levigne[3] · Martine Wallon[3] · Jose G. Montoya[4,7] · Hongjie Dai[1]

Received: 10 December 2018 / Accepted: 13 January 2019 / Published online: 30 January 2019

Abstract

Sampling the blood compartment by an invasive procedure such as phlebotomy is the most common approach used for diagnostic purposes. However, phlebotomy has several drawbacks including pain, vasovagal reactions, and anxiety. Therefore, alternative approaches should be tested to minimize patient's discomfort. Saliva is a reasonable compartment; when obtained, it generates little or no anxiety. We setup a multiplexed serology assay for detection of *Toxoplasma gondii* IgG and IgM, rubella IgG, and CMV IgG, in serum, whole blood, and saliva using novel plasmonic gold (pGOLD) chips. pGOLD test results in serum, whole blood, and saliva were compared with commercial kits test results in serum. One hundred twenty serum/saliva sets (Lyon) and 28 serum/whole blood/saliva sets (Nice) from France were tested. In serum and whole blood, sensitivity and specificity of multiplex *T. gondii*, CMV, and rubella IgG were 100% in pGOLD when compared to commercial test results in serum. In saliva, sensitivity and specificity for *T. gondii* and rubella IgG were 100%, and for CMV IgG, sensitivity and specificity were 92.9% and 100%, respectively, when compared to commercial test results in serum. We were also able to detect *T. gondii* IgM in saliva with sensitivity and specificity of 100% and 95.4%, respectively, when compared to serum test results. Serological testing by multiplex pGOLD assay for *T. gondii*, rubella, and CMV in saliva is reliable and likely to be more acceptable for systematic screening of pregnant women, newborn, and immunocompromised patients.

Keywords Saliva · Toxoplasmosis · Cytomegalovirus · Rubella · Plasmonic gold chips · Multiplexed serologies

Introduction

Laboratory testing of the blood compartment is essential in today's health care environments for a vast majority of decisions that are made every day regarding patients' diagnosis and treatments. Blood samples are primarily obtained by phlebotomy. However, phlebotomy is an invasive procedure with several associated drawbacks

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10096-019-03487-1) contains supplementary material, which is available to authorized users.

✉ Christelle Pomares
pomares.c@chu-nice.fr

✉ Jose G. Montoya
gilberto@stanford.edu

✉ Hongjie Dai
hdai1@stanford.edu

[1] Department of Chemistry, Stanford University, William Keck Science Building rm 125, Stanford, CA 94305-5080, USA

[2] Service de Parasitologie Mycologie, Centre Hospitalier Universitaire de Nice, INSERM, U1065, Centre Méditerranéen de Médecine Moléculaire, Faculté de Médecine, Virulence microbienne et signalisation inflammatoire – Université de la Côte d'Azur, CS 23079 Nice, cedex 3, France

[3] Institut de Parasitologie et de Mycologie Médicale, Hôpital de la Croix Rousse, Lyon, France

[4] Palo Alto Medical Foundation Toxoplasma Serology Laboratory, Palo Alto, CA, USA

[5] Service de Virologie, Centre Hospitalier Universitaire de Nice, Nice, France

[6] Emerging Pathogens Laboratory - Fondation Mérieux, Centre, International de Recherche en Infectiologie (CIRI) Inserm U1111, CNRS UMR5308, ENS de Lyon, UCBL1, Lyon, France

[7] Division of Infectious Diseases, Department of Medicine, Stanford University, 300 Pasteur Dr, MC 5107, Stanford, CA 94305, USA

including pain, anxiety, and vasovagal reactions. In addition, trained personnel and sterile equipment are required in order to perform sampling of the blood compartment. Moreover, for infants, young children, and persons with severe belonephobia, collection of blood can be a real challenge and result in bruises. For patients requiring repeated testing, serial sampling can become even more challenging as veins can be damaged from one sampling to another. Furthermore, during the blood sampling procedure, there is the potential risk of accidental blood exposure for health care personnel. Thus, sampling of body compartments other than blood and easier collection should be explored for diagnostic purposes. Oral fluid appears to be an appealing option since accessing this body compartment does not have any of the drawbacks associated with venipuncture, requires less manipulation by health care personnel, and does not clot [1]. Oral fluid is composed of saliva and crevicular fluid and contains IgG, IgM, and IgA; concentration of IgG and IgM is lower than in serum [1, 2]. Thus, detection of pathogen-specific antibodies in saliva represents a promising tool and a new challenge for future developments in serological testing. Despite the simplicity and advantages of sampling oral fluids, very few commercially available assays are using oral fluids for serological diagnostic purposes [3]. Most of the available saliva assays primarily use ELISA or ELISA-like methods and are aimed at detecting only one antibody at a time.

Recently, our team setup a *T. gondii* multiplex IgG, IgM, IgA assay on plasmonic gold (pGOLD) platform in serum that performed with outstanding sensitivity and specificity when compared to individual *T. gondii* IgG, IgM, and IgA test results obtained at the Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory (PAMF-TSL), a reference laboratory for toxoplasmosis in the USA [4–6]. Since pathogens other than *T gondii* can also result in serious congenital infection, we have been researching assays with multiplexing capacity for the simultaneous detection of pathogens capable of mother to child transmission [7–9]. As an initial exploratory step and proof of concept, we decided to setup such assay for *T. gondii*, CMV, and rubella [7–9]. When infection by one of these 3 pathogens occurs during pregnancy, symptoms may not be present or are non-specific; therefore, diagnosis of maternal infection primarily relies on serological tests in all women. A multiplexed serology assay could greatly improve the workup and follow-up of pregnant women and their infants. In addition, by using a multiplexed technology, the screening could be cost effective as one run gives results of multiple serologies.

The purpose of this study was to adapt the innovative technology of pGOLD to setup a multiplexed serology for detection of IgG and IgM against *T. gondii*, and IgG against rubella and CMV in serum, whole blood, and saliva.

Methods

Sample collection

Sera and paired saliva from Lyon (France) were obtained from 120 patients aged from 0 to 66 years old. Saliva was collected using two microsponges as previously described [10]. The Lyon specimens had been already collected and had been included in patient's outpatient follow-up. Sera from these patients were tested for *T. gondii* serology on a commercial serological platform.

Sera, whole blood, and saliva from Nice (France) were prospectively collected from 28 adult patients aged from 21 to 61. Saliva was collected by spitting in a tube. Sera were tested for *T. gondii*, CMV, and rubella serologies on a commercial serological platform.

Serology testing on commercial assays

T. gondii serology was performed by detecting IgG and IgM with Architect and AxSYM Abbott IgG and IgM assays (Abbott Laboratories, USA). CMV IgG serology was performed on Liaison XL platform (DiaSorin, Italy) and rubella IgG serology was performed on Vidas platform (BioMérieux, France). According to the manufacturer's instructions, results of IgG and IgM *T. gondii* assays were interpreted as positive, gray zone, and negative respectively: IgG Abbott ≥ 3; 2.9–1.6; < 1.6; and IgM Abbott ≥ 0.60; 0.59–0.50; < 0.50. In our sample set, the quantification of Abbott IgG test results ranges from 3.90 to 973.70 UI/ml. CMV and rubella IgG assays were interpreted as positive, gray zone, and negative respectively: CMV IgG ≥ 15; 14.9–11; < 11; and IgG rubella: ≥ 15; 15–10; < 10.

For one patient, IgG test result on Architect was in gray zone. In order to clarify the interpretation of the serology, a Dye test was performed at the PAMF-TSL and the IgG result was considered positive as the Dye test result was 128 [11].

Of the 148 serum samples (120 from Lyon and 28 from Nice) tested for *T. gondii* serology, 70 had negative and 78 had positive Abbott IgG test results. For IgM tests, 17 had positive Abbott IgM and 131 had negative IgM test results.

Of the 28 serum samples tested on commercial assays, 27 and 1 test results were positive and negative, respectively, for IgG CMV; and 14 and 14 tests results were positive and negative, respectively, for IgG rubella.

This study was approved by the local ethical committees for the prospective collection of samples (Comité de protection des personnes (CPP) Teaching hospitals of Lyon and Nice, France).

Multiplexed antigen microarray fabrication on pGOLD biochip

A plasmonic gold film composed of tortuous gold nanoislands was fabricated on glass slides through a solution phase growth method as previously described, resulting in a surface packed with gold nanoislands with plasmon resonance in the NIR (near-infrared) region and abundant nanoscale gaps [5].

The pGOLD biochips (Nirmidas Biotech) were coated with the antigens of interest. The antigens were prepared and delivered to pGOLD (Nirmidas) biochips using GeSiM NanoPlotter 2.1 at the following concentration: 0.15 mg/ml CMV antigen (CMV Concentrate Antigen, Meridian Life Science, Inc.), 0.33 mg/ml rubella antigen (rubella virus Antigen, Meridian Life Science, Inc.), and *T. gondii* antigen (provided by PAMF-TSL). The selected antigens were printed in triplicate and were used to capture *T. gondii* IgG and IgM antibodies, CMV IgG, and rubella IgG in serum, whole blood, and saliva.

Microarray consisted of three microarray spots of each of the CMV, rubella, and *T. gondii* antigens was fabricated. Microarray followed a 3 × 3 layout (Fig. 1a), spot diameter was ~400 μm, and the distance between spots was 1000 μm. Sixteen identical microarrays were formed on each pGOLD biochip. The fabricated biochips were vacuum sealed and stored at − 20 °C before use.

Secondary antibody fabrication

The pGOLD platform enhances the fluorescence detection in the 500- to 900-nm visible-to-NIR spectral window. Thus, multiple dyes with non-overlapping emission spectra such as IRDye800 and IRDye680 could be simultaneously detected [12–15]. To reduce the high background noise signals that are usually associated with saliva assays and hence hinder the diagnostic value of salivary fluid, we conjugated IRDye800 CW NHS ester and IRDye680 LT NHS ester (LI-COR Biosciences) to the secondary antibodies. These fluorescence signals from these dyes are enhanced 50- to 100-fold fluorescence by the underlying plasmonic gold chips [13]. This leads to boosted signal-to-background ratio and contributes to the accurate reporting of the IgG and IgM antibody signals. Secondary antibodies consisted of anti-human IgG and IgM purchased from Vector Laboratories. After conjugation, we obtained IRDye800–anti-human IgG and IRDye680–anti-human IgM. The multicolor capacity allowed simultaneous detection of the IgG and IgM antibody subtypes in the same sample with high signal-to-noise ratios and a broad ~ 5-log

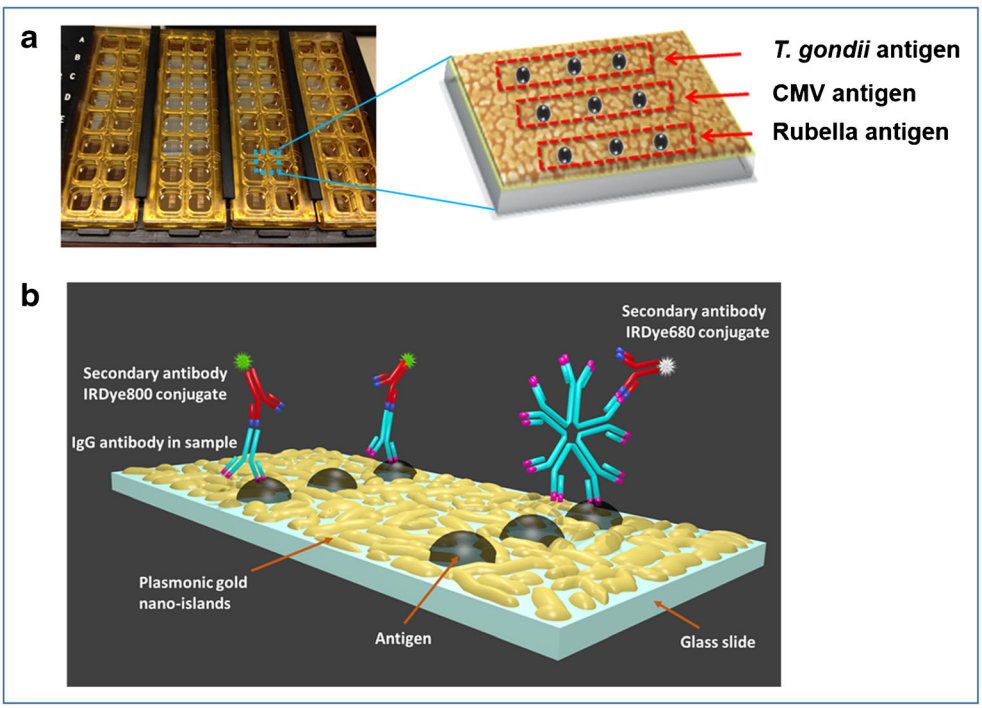

Fig. 1 Multiplexed *T. gondii*/CMV/rubella antigen microarray on plasmonic gold biochip. **a** The plasmonic gold biochips were integrated in a 4-chip module in which 16 identical microarrays on each biochip were separated into 16 wells to process 16 different samples. Each well has identical antigen microarray. For each microarray, *T. gondii*, CMV, and rubella antigen spots were immobilized on pGOLD in triplicate. **b** During testing, human sample is applied to the microarray, where human IgG and IgM antibodies against *T. gondii* antigens or human IgG antibodies against CMV and/or rubella, if present, will be captured on corresponding antigen spots. After a washing step, a mixture of anti-human IgM-IRDye680 conjugate and anti-human IgG-IRDye800 conjugate is applied to the microarray to label captured human IgM antibody with IRDye680 and human IgG antibody with IRDye800. Then, the plasmonic gold biochip is scanned and fluorescent signals are analyzed

dynamic range of the signals for both antibody subtypes [5, 6, 16]

Multiplexed assay process

The assay system was composed of the printed antigen array on a pGOLD biochip, biochip frame, buffers, plate washer, and a dual-channel scanner.

The fabricated chips were integrated in a 4-chip module (ProPlate; Grace Bio-Labs) in which 16 identical microarrays on each biochip were separated into 16 wells to process 16 different samples (Fig. 1a). All biochips were firstly blocked with 5% bovine serum albumin (BSA, Sigma-Aldrich) in phosphate buffered saline (1× PBS, GE Healthcare Life Sciences) for 10 min. For the detection of *T. gondii* IgG and IgM, each of the well was then incubated with human saliva (25 μl of saliva diluted 4 times in fetal bovine serum) or matched human sera (1 μl of sera diluted 400 times in fetal bovine serum) for 25 min.

For the multiplexed detection of *T. gondii* IgG and IgM, CMV, and rubella IgG, each of the well was incubated with human saliva, matched human sera, or matched whole blood sample for 25 min. Dilutions were performed as described above and whole blood samples were diluted as sera.

The biochips were subsequently incubated with the mixture of 2 nM IRDye800-labeled anti-human IgG secondary antibody and 2 nM IRDye680-labeled anti-human IgM secondary antibody for 15 min (Fig. 1b). Each well was washed three times with PBST (PBS–0.05% Tween 20, Sigma-Aldrich) between each incubation procedure. Fourteen human samples, together with two reference samples, were applied to each biochip. Reference samples consisted of one serum sample positive for *T. gondii*, CMV, and rubella IgG and positive for *T. gondii* IgM, and one serum sample with negative IgG for the three serologies and negative for *T. gondii* IgM.

Data analysis

After the assay process, LI-COR Odyssey dual-channel (700-nm/800-nm) scanner (LI-COR Biosciences) was used to scan each biochip for the IRDye800–anti-human IgG and IRDye680–anti-human IgM signals. IRDye680 and IRDye800 fluorescence images were generated, and the median fluorescence signal for each channel on each microarray spot was quantified by GenePix 6.1 software. For each sample, each antigen and each channel, the average of the three mean fluorescence intensities (MFI) for three spots was calculated and normalized by reference positive sample.

The cutoff of the three serologies (*T. gondii*, CMV, and rubella) on the pGOLD biochip was determined by mean Ig levels of non-infected samples + 3 standard deviations (s.d.). This method resulted in the optimal combination of sensitivity and specificity of the multiplexed assay on pGOLD.

Statistical analyses

Measurements were performed three times for all values presented in this work, and the average is presented with error bars in relevant figures. The error bars demonstrate the standard deviation of three measurements for each value. Comparisons of *T. gondii* IgG and *T. gondii* IgM levels measured for different patient cohorts using serum and saliva samples were assessed using an unpaired two-tailed Student's *t* test. Results were considered to be significant for $p < 0.01$. The K coefficient (Cohen's kappa coefficient) was used to compare the results of pGOLD to those from the commercial test results. In all box plots, measurement data (points) with median value (center line) and average value (center small box), 1%, 25%, 75%, and 99% value lines (box lines) are presented.

Results

Saliva detection of *T. gondii* IgG and IgM antibodies on plasmonic gold platform

We tested the serum and saliva samples of 148 subjects on pGOLD chips and measured *T. gondii*-specific IgG and IgM antibodies in this multiplexing platform. Tests results on pGOLD chips were compared with Abbott IgG and IgM tests results.

In serum, levels of IgG (Fig. 2a) and IgM (Fig. 2d) tested on plasmonic gold chips were significantly higher in subjects tested positive for IgG and IgM compared to those tested negative on commercial assays ($p < 0.00001$). *T. gondii* IgG cutoff of 0.3 was able to differentiate 78/78 (100%) individuals with positive Abbott IgG from the IgG-negative subjects (Fig. 2a and Fig. S1). With a cutoff of 1.5 (Fig. 2d), the *T. gondii* IgM test afforded 94.1% sensitivity and 97.7% specificity (Table 1) as it identified 16/17 individuals with positive Abbott IgM and 128/131 subjects with negative Abbott IgM test results (Fig. S2).

For saliva detection, the *T. gondii* IgG (Fig. 2b) and IgM (Fig. 2e) levels detected in subjects with positive Abbott IgG and IgM test results were also substantially higher than those detected in *T. gondii* IgG- and IgM-negative subjects ($p < 0.0001$). The *T. gondii* IgG test in saliva afforded very high sensitivity of 96.1% with 93.0% specificity (Table 1). At a cutoff of 0.35, 74/78 of the positive Abbott IgG test results were successfully identified (Fig. 2b and Fig. S3). The *T. gondii* IgM test offered complete positive percentage agreement and very high specificity of 95.4% as 125/131 of the negative Abbott IgM test results could be differentiated at a cutoff of 0.32 (Table 1, Fig. 2e and Fig. S4).

The correlation coefficient between saliva and serum detection on pGOLD for *T. gondii* IgG and IgM was 0.88 and

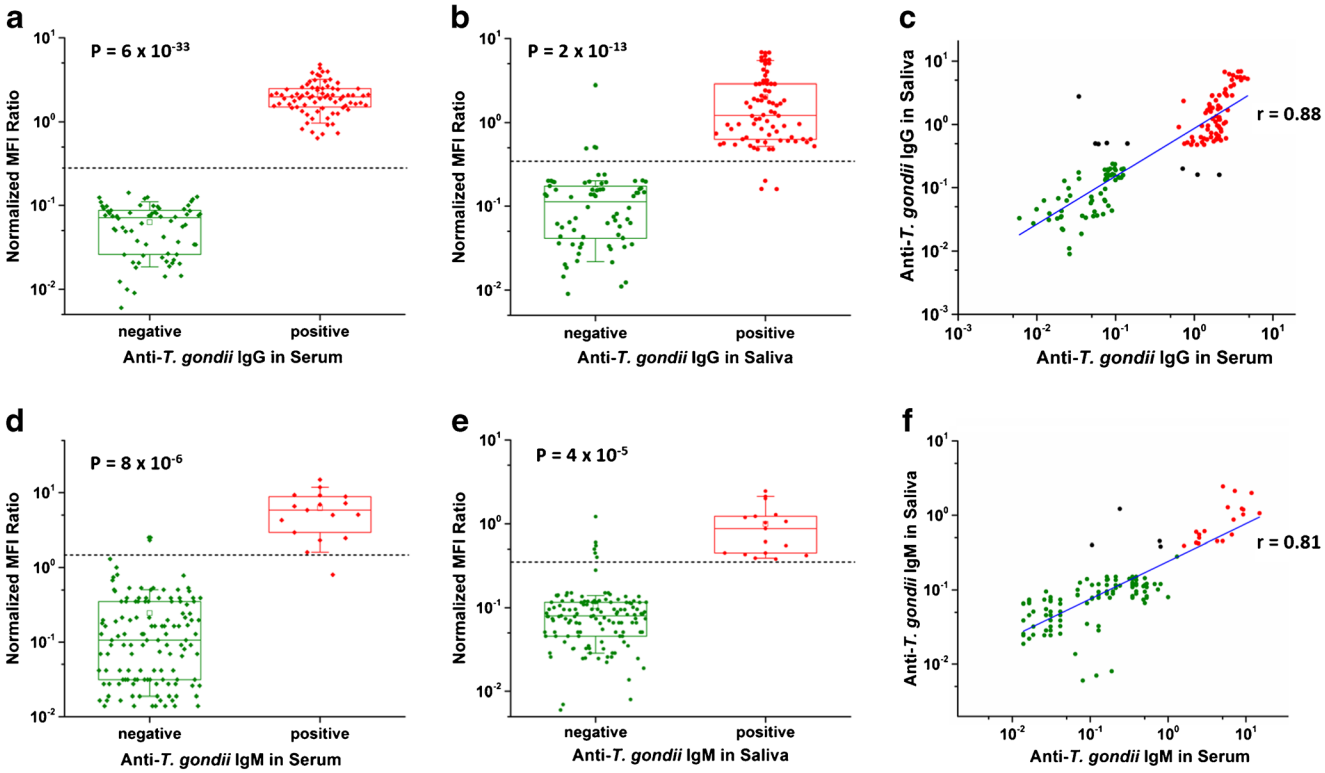

Fig. 2 Detection of *T. gondii*-specific IgG and IgM antibodies in 148 subjects using saliva on plasmonic gold chips afforded serum detection precision. Boxplots showing IgG antibody against *T. gondii* antigen in **a** serum and **b** saliva. **c** Correlation between saliva and serum detection of *T. gondii* IgG antibodies. Boxplots showing IgM antibody against *T. gondii* antigen in **d** serum and **e** saliva. **f** Correlation between saliva and serum detection of *T. gondii* IgM antibodies. All signal levels are normalized by a reference sample

0.81 respectively (significance level $p < 0.0001$) (Fig. 2c and f) which highlighted an almost perfect agreement between serum and saliva test results on the pGOLD platform.

Simultaneous detection of multiple infectious diseases in saliva

The diagnostic value of saliva for multiple infectious diseases in a multiplex setting was investigated by incubating matched serum, saliva, and whole blood samples of subjects over pGOLD chips printed with *T. gondii*, CMV, and rubella antigens with known spatial orientation (Fig. 3a). For individuals who had single type of infection, fluorescent signals were only detectable on the antigen spots corresponding to that particular infection and not on any other spots (Fig. 3a). We did not observe any cross-reactivity issues, indicating a high specificity of our multiplexed assays.

In saliva, our cohort of 28 subjects was selected for the multiplexed detection of *T. gondii* IgG and IgM, CMV IgG, and rubella IgG, and their test results on pGOLD platform were compared to their serological status based on the commercial tests results in serum. Here, the *T. gondii* IgG and rubella IgG detection in saliva afforded 100% specificity and sensitivity (Fig. 3b and e and Table 2). *T. gondii* IgM detection in saliva successfully identified 27/28 of the Abbott IgM-negative subjects (Fig. 3c) offering a high specificity of 96.4% with 100% positive predictive value (Table 2). The CMV IgG detection in saliva in this multiplexed assay afforded 100% specificity and positive predictive value with 92.9% sensitivity and 93.3% negative predictive value (Fig. 3d and Table 2).

Table 1 *T. gondii* infection diagnostic table summarizing *T. gondii* IgG/IgM test results in 148 subjects

		Sensitivity (%)	Specificity (%)	K coefficient	PPV (%)	NPV (%)
T. gondii IgG	Serum	100	100	1	100	100
	Saliva	96.1	93.0	0.892	93.7	95.7
T. gondii IgM	Serum	94.1	97.7	0.874	84.2	99.2
	Saliva	100	95.4	0.827	73.9	100

Sensitivity, specificity, Kappa coefficient, positive predictive value (PPV), and negative predictive value (NPV) of *T. gondii* IgG and IgM tests on plasmonic gold chips in matched serum and saliva samples

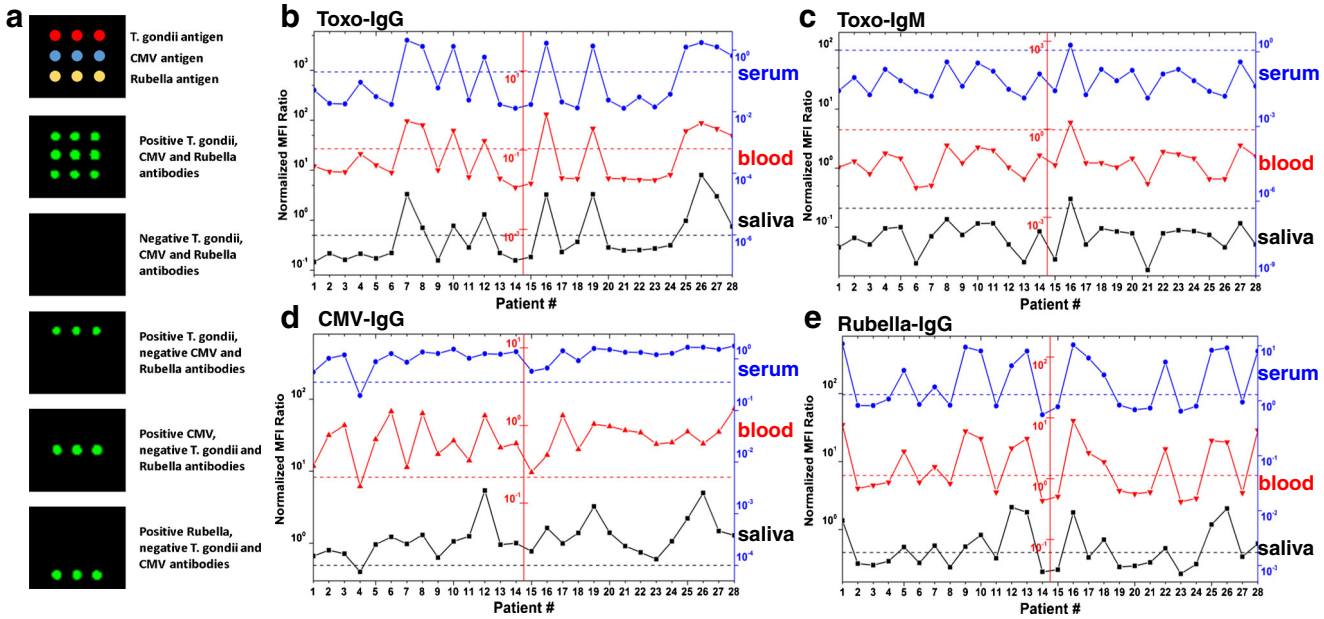

Fig. 3 Multiplexed panel for the simultaneous detection of three infectious diseases in 28 subjects. *T. gondii* IgG and IgM, CMV IgG, and rubella IgG antibodies in 28 matched serum, saliva, and whole blood samples. **a** Scanning images showing positive detection signals vs negative signals in serum, blood and saliva. Saliva and whole blood afforded comparable sensitivity and specificity to serum in the multiplexed detection of **b** *T. gondii* IgG, **c** *T. gondii* IgM, **d** CMV IgG, and **e** Rubella IgG antibodies. Dotted lines represent cutoff levels for each detection

Furthermore, almost perfect correlation was observed between serum and saliva detection for all antibodies in this multiplexed assay (Fig. 3b to e). Finally, complete agreement in detection results was observed between the matched whole blood and serum samples of these 28 subjects.

These test results demonstrated that using the multiplex pGOLD chips with saliva and whole blood provided the same detection precision as serum for the detection of multiple pathogens including *T gondii*, CMV, and rubella.

All samples were tested 3 times against the three antigens in independent experiments on various days and on different gold chips. Excellent reproducibility of the assay results on the plasmonic gold chips was achieved; the coefficient of variation was < 13%.

Table 2 Multiplexed detection of *T. gondii*, CMV and rubella infections in 28 subjects using matched saliva, serum and whole blood

		Sensitivity (%)	Specificity (%)	K coefficient	PPV (%)	NPV (%)
T. gondii IgG	Serum	100	100	1	100	100
	Saliva	100	100	1	100	100
	Whole blood	100	100	1	100	100
T. gondii IgM	Serum	NA	96.4	NA	NA	100
	Saliva	NA	96.4	NA	NA	100
	Whole blood	NA	96.4	NA	NA	100
CMV IgG	Serum	100	100	1	100	100
	Saliva	92.9	100	0.929	100	93.3
	Whole blood	100	100	1	100	100
Rubella IgG	Serum	100	100	1	100	100
	Saliva	100	100	1	100	100
	Whole blood	100	100	1	100	100

Sensitivity, specificity, Kappa coefficient, positive predictive value (PPV), and negative predictive value (NPV) of *T. gondii* IgG and IgM, CMV IgG, and rubella IgG antibody test results on plasmonic gold chips in saliva and whole blood in comparison with results of commercially available test results. Since all 28 subjects were toxo-IgM negative, only specificity and NPV of toxo-IgM tests could be calculated and these values are 96.4% and 100% respectively

Discussion

In this study, we report a novel use of the multiplex pGOLD platform. We applied this plasmonic gold nanotechnology to the diagnosis of *T. gondii*, rubella virus, and CMV infections. This technology has been already successfully used for *T. gondii* IgG, IgM, and IgA detection in serum with excellent correlation with Dye test results and clinical interpretation of *T. gondii* serological test results [5, 6]. Infection by *T. gondii*, rubella, and CMV during pregnancy could result in severe morbidity and mortality of the offspring. Infection by one of these three pathogens is frequently asymptomatic or, if symptomatic, symptoms are non-specific [7–9]. Therefore, the diagnosis primarily relies on serological testing. Systematic serological screening during gestation has been demonstrated to be beneficial for congenital infections such as *T. gondii* [17–23]. For CMV, knowledge of the serological status leads to a reduction of seroconversion rate during pregnancy [24]. In addition, if CMV infection occurs during pregnancy, some studies have shown that treatment has been proven to reduce severe fetal outcome [24–26]. However, current data on antiviral efficacy and safety profiles during pregnancy are limited and treatments are not routinely recommended [26]. Rubella screening allows identification of pregnant women who are not immunized and are susceptible to contract rubella. Non-immunized pregnant women will be informed of preventive measures to avoid infection during first trimester of pregnancy [7, 27]. Moreover, in this population, rubella vaccine is proposed in the postnatal period in order to protect future pregnancies [7, 27]. Despite all the advantages listed above, serological screening for *T. gondii*, CMV, and rubella is not worldwide implemented during pregnancy, and when performed, the screening is not systematic for all the pathogens that can cause congenital infection. In order to be implemented on a large scale, screening should be cost effective and constraints should be limited for a greater compliance with recommendations.

The pGOLD platform, with its ability to enhance around 100-fold, the fluorescence signal has several advantages [14–16]. Our data suggest that three different matrices (serum, whole blood, and saliva) can successfully be used and with utilization of very small sample volumes (~ 1 μl of serum or whole blood and ~ 25 μl of saliva). In our study, serum was used as the reference matrix. Whole blood has the benefit that it does not require centrifugation. Moreover, due to the small amount of whole blood required, we can image in a near future to realize test on whole blood collected by finger prick avoiding phlebotomy. For saliva, the collection through a non-invasive procedure made this matrix a good candidate for patients to perform themselves the sampling with cheap equipment. This matrix has already been described as a good candidate for the workup and follow-up of infants at risk of congenital toxoplasmosis [10]. In addition, saliva sampling does not require trained staff. Because saliva is easy to obtain, it could increase compliance and prevent accidental exposure to blood. Thus, saliva could be a good alternative to serum to implement screening for *T. gondii*, CMV, and rubella at a lower cost compared to serum.

The multiplex pGOLD platform allowed simultaneous (multiplex) detection of *T. gondii*, CMV, and rubella serologies. In serum and whole blood, sensitivity and specificity of multiplex *T. gondii*, CMV, and rubella IgG by the pGOLD assay were 100% when compared to commercial test results. In saliva, despite the low IgG concentration, sensitivity and specificity for *T. gondii* and rubella IgG were 100%, and for CMV IgG, sensitivity and specificity were 92.9% and 100%, respectively, when compared to commercial test results in serum. We were also able to detect *T. gondii* IgM in saliva with specificity 95.4%. One sample had false-positive test result for *T. gondii* IgM detection. This result was very close to the defined cutoff. However, our results were obtained on limited sample set. Plasmonic GOLD assay performed on an extended sample set may help to define a better cutoff and or a gray zone to improve interpretation of the serology.

In terms of public health, Stillwagon et al. have demonstrated the cost effectiveness of a monthly serological screening for *T. gondii* in the USA [28]. With its multiplex capacities, the pGOLD platform for *T. gondii*, CMV, and rubella could significantly reduce cost of screening and therefore should lead public health authorities to reconsider the recommendations of serological screening for pregnant women. In addition, the very good results obtained with saliva allow the use of this non-invasive matrix to perform systematic screening. It would become therefore simpler with less constraint for the patient which it is very likely to have a positive impact on compliance with universal screening programs. With saliva, cost of pre-analytic process is also reduced. All these considerations taken together, serological screening by the multiplex pGOLD assay for *T. gondii*, rubella, and CMV on saliva could transform mass screening into affordable and acceptable for pregnant women and infants. Further studies are warranted to broaden the scope of pGOLD to other congenital infections such as hepatitis B, HIV, Syphilis, and Zika.

Funding information Christelle Pomares received a grant from the "Philippe Foundation Inc.," the "Association des amis de la Faculté de Médecine de Nice," and from REDPIT (Recherche Et Developpement En Pathologie Infectieuse Et Tropicale) association. These associations allowed a personal financial support during the post-doctoral period in the USA.

Compliance with ethical standards

This study was approved by the local ethical committees for the prospective collection of samples (Comité de protection des personnes (CPP) Teaching hospitals of Lyon and Nice, France).

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DTW (2013) Salivary biomarkers: toward future clinical and diagnostic utilities. Clin Microbiol Rev 26:781–791. https://doi.org/10.1128/CMR.00021-13
2. Exum NG, Pisanic N, Granger DA, Schwab KJ, Detrick B, Kosek M et al (2016) Use of pathogen-specific antibody biomarkers to estimate waterborne infections in population-based settings. Curr Environ Health Rep 3:322–334. https://doi.org/10.1007/s40572-016-0096-x
3. Stekler JD, Ure G, O'Neal JD, Lane A, Swanson F, Maenza J et al (2016) Performance of determine combo and other point-of-care HIV tests among Seattle MSM. J Clin Virol 76:8–13. https://doi.org/10.1016/j.jcv.2015.12.011
4. Augustine SAJ (2016) Towards universal screening for toxoplasmosis: rapid, cost-effective, and simultaneous detection of anti-*Toxoplasma* IgG, IgM, and IgA antibodies by use of very small serum volumes. J Clin Microbiol 54:1684–1685. https://doi.org/10.1128/JCM.00913-16
5. Li X, Pomares C, Gonfrier G, Koh B, Zhu S, Gong M et al (2016) Multiplexed anti-*Toxoplasma* IgG, IgM, and IgA assay on plasmonic gold chips: towards making mass screening possible with dye test precision. J Clin Microbiol 54:1726–1733. https://doi.org/10.1128/JCM.03371-15
6. Pomares C, Zhang B, Arulkumar S, Gonfrier G, Marty P, Zhao S et al (2017) Validation of IgG, IgM multiplex plasmonic gold platform in French clinical cohorts for the serodiagnosis and follow up of *Toxoplasma gondii* infection. Diagn Microbiol Infect Dis [cited 2016 28]; http://www.sciencedirect.com/science/article/pii/S0732889316302863. https://doi.org/10.1016/j.diagmicrobio.2016.09.001
7. Yazigi A, Pecoulas AED, Vauloup-Fellous C, Grangeot-Keros L, Ayoubi J-M, Picone O (2016) Fetal and neonatal abnormalities due to congenital rubella syndrome: a review of literature. J Matern Fetal Neonatal Med 22(0):1–5. https://doi.org/10.3109/14767058.2016.1169526
8. Hui L, Wood G (2015) Perinatal outcome after maternal primary cytomegalovirus infection in the first trimester: a practical update and counseling aid. Prenat Diagn 35:1–7. https://doi.org/10.1002/pd.4497
9. Picone O, Grangeot-Keros L, Senat M, Fuchs F, Bouthry E, Ayoubi J et al (2016) Cytomegalovirus non-primary infection during pregnancy. Can serology help with diagnosis? J Matern Fetal Neonatal Med 5:1–4. https://doi.org/10.3109/14767058.2016.1169521
10. Chapey E, Meroni V, Kieffer F, Bollani L, Ecochard R, Garcia P et al (2015) Use of IgG in oral fluid to monitor infants with suspected congenital toxoplasmosis. Clin Vaccine Immunol 22:398–403. https://doi.org/10.1128/CVI.00552-14
11. Sabin AB, Feldman HA (1948) Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (toxoplasma). Science 108:660–663. https://doi.org/10.1126/science.108.2815.660
12. Zhang B, Yang J, Zou Y, Gong M, Chen H, Hong G et al (2014) Plasmonic micro-beads for fluorescence enhanced, multiplexed protein detection with flow cytometry. Chem Sci 26(5):4070–4075. https://doi.org/10.1039/C4SC01206B
13. Tabakman SM, Lau L, Robinson JT, Price J, Sherlock SP, Wang H et al (2011) Plasmonic substrates for multiplexed protein microarrays with femtomolar sensitivity and broad dynamic range. Nat Commun 2:466. https://doi.org/10.1038/ncomms1477
14. Koh B, Li X, Zhang B, Yuan B, Lin Y, Antaris AL et al (2016) Visible to near-infrared fluorescence enhanced cellular imaging on plasmonic gold chips. Small 27(12):457–465. https://doi.org/10.1002/smll.201502182
15. Zhang B, Price J, Hong G, Tabakman SM, Wang H, Jarrell JA et al (2013) Multiplexed cytokine detection on plasmonic gold substrates with enhanced near-infrared fluorescence. Nano Res 6:113–120. https://doi.org/10.1007/s12274-012-0286-2
16. Zhang B, Kumar RB, Dai H, Feldman BJ (2014) A plasmonic chip for biomarker discovery and diagnosis of type 1 diabetes. Nat Med 20:948–953. https://doi.org/10.1038/nm.3619
17. Pomares C, Montoya JG (2016) Laboratory diagnosis of congenital toxoplasmosis. J Clin Microbiol 54:2448–2454. https://doi.org/10.1128/JCM.00487-16
18. Cortina-Borja M, Tan HK, Wallon M, Paul M, Prusa A, Buffolano W et al (2010) Prenatal treatment for serious neurological sequelae of congenital toxoplasmosis: an observational prospective cohort study. PLoS Med 7. https://doi.org/10.1371/journal.pmed.1000351
19. Hotop A, Hlobil H, Gross U (2012) Efficacy of rapid treatment initiation following primary *Toxoplasma gondii* infection during pregnancy. Clin Infect Dis 54:1545–1552. https://doi.org/10.1093/cid/cis234
20. Kieffer F, Wallon M, Garcia P, Thulliez P, Peyron F, Franck J (2008) Risk factors for retinochoroiditis during the first 2 years of life in infants with treated congenital toxoplasmosis. Pediatr Infect Dis J 27:27–32. https://doi.org/10.1097/INF.0b013e318134286d
21. Prusa A-R, Kasper DC, Pollak A, Gleiss A, Waldhoer T, Hayde M (2015) The Austrian Toxoplasmosis Register, 1992-2008. Clin Infect Dis 15(60):e4–e10. https://doi.org/10.1093/cid/ciu724
22. Wallon M, Peyron F, Cornu C, Vinault S, Abrahamowicz M, Kopp CB et al (2013) Congenital *Toxoplasma* infection: monthly prenatal screening decreases transmission rate and improves clinical outcome at age 3 years. Clin Infect Dis 56:1223–1231. https://doi.org/10.1093/cid/cit032
23. SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group, Thiébaut R, Leproust S, Chêne G, Gilbert R (2007) Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. Lancet 369:115–122. https://doi.org/10.1016/S0140-6736(07)60072-5
24. Carlson A, Norwitz ER, Stiller RJ (2010) Cytomegalovirus infection in pregnancy: should all women be screened? Rev Obstet Gynecol 3:172–179
25. Rawlinson WD, Hamilton ST, van Zuylen WJ (2016) Update on treatment of cytomegalovirus infection in pregnancy and of the newborn with congenital cytomegalovirus. Curr Opin Infect Dis 29:615–624. https://doi.org/10.1097/QCO.0000000000000317
26. Rawlinson WD, Boppana SB, Fowler KB, Kimberlin DW, Lazzarotto T, Alain S et al (2017) Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. Lancet Infect Dis 17:e177–e188. https://doi.org/10.1016/S1473-3099(17)30143-3
27. Bouthry E, Picone O, Hamdi G, Grangeot-Keros L, Ayoubi J-M, Vauloup-Fellous C (2014) Rubella and pregnancy: diagnosis, management and outcomes. Prenat Diagn 34:1246–1253. https://doi.org/10.1002/pd.4467
28. Stillwaggon E, Carrier CS, Sautter M, McLeod R (2011) Maternal serologic screening to prevent congenital toxoplasmosis: a decision-analytic economic model. PLoS Negl Trop Dis 5:e1333. https://doi.org/10.1371/journal.pntd.0001333