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Title:

Mycoplasma genitalium detection in urogenital specimens from symptomatic and asymptomatic men and women using the cobas TV/MG test

Running title (max 54 characters):

Detection of Mycoplasma using the cobas® TV/MG test

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ABSTRACT

Mycoplasma genitalium (MG) infections are a growing concern within the field of sexually transmitted infections. However, diagnostic assays for MG have been limited in the United States (US). As most infections are asymptomatic, individuals can unknowingly pass the infection on and the prevalence is likely to be underestimated. Diagnosis of MG infection is recommended using a nucleic acid test. This multicenter study assessed the performance of the cobas® TV/MG assay (cobas) for the detection of MG, using 22,150 urogenital specimens from both symptomatic and asymptomatic men and women collected at geographically diverse sites across the US. The performance was compared to a reference standard of three laboratory-developed tests (LDTs). The specificity of the cobas assay for MG ranged from 96.0% to 99.8% across symptomatic and asymptomatic men and women. The sensitivity in female vaginal swabs and urine samples was 96.6% (95% confidence interval [CI] 88.5–99.1%) and 86.4% (95% CI 75.5–93.0%), respectively. The sensitivity in male urine and meatal swab samples was 100% (95% CI 94.0–100%) and 85.0% (95% CI 73.9–91.9%), respectively. This study demonstrated that the cobas assay was highly sensitive and specific in all relevant clinical samples for the detection of MG.

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61 INTRODUCTION

62 *Mycoplasma genitalium* (MG) is a sexually transmitted infection (STI) which has been
63 associated with urethritis, cervicitis, pelvic inflammatory disease, and male and female
64 infertility in epidemiologic studies (1-8). The prevalence of MG infection varies depending on
65 the geographical region, gender, and the presence of risk factors. In the general population,
66 it is estimated to range from 1% to 2% (9-12), and in patients attending sexual health
67 clinics the estimates range from 3.3% to 38% (2, 13-18).

68

69 Many MG infections are asymptomatic and, therefore, it is possible for individuals to
70 unknowingly transmit the infection to their sexual partners (19-21). Asymptomatic infections
71 can lead to pelvic inflammatory disease, which is associated with serious long-term
72 sequelae, including ectopic pregnancy, infertility, and pelvic/abdominal pain (3, 22, 23). The
73 extent to which these sequelae can be attributed to asymptomatic MG infections is
74 unknown, in part due to a lack of sensitive diagnostic tools. MG is difficult to culture,
75 typically requiring several weeks or months, meaning that, historically, MG infections were
76 rarely diagnosed and it was difficult to estimate their prevalence (24, 25). MG infections can
77 now be rapidly detected using nucleic acid amplification tests (NAATs). Accurate detection of
78 MG is important for treatment of symptomatic infections, as many strains of MG have
79 developed resistance to the empiric treatments for urethritis or cervicitis (3, 8, 13, 19, 25-
80 29).

81

82 Despite its relatively high prevalence compared with other STIs, such as gonorrhea,
83 screening for MG infections in asymptomatic individuals is not recommended, due to our
84 limited understanding of the consequences of asymptomaic infection and the need for
85 antimicrobial stewardship (i.e. not treating infections that may naturally clear without harm).
86 Only targeted testing of symptomatic or high-risk individuals is recommended by the

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currently published guidelines for STI screening and treatment (3, 25). In the US, there are currently only two FDA-approved diagnostic tests for the detection of MG in urogenital specimens: the Aptima™ *Mycoplasma genitalium* (APT MG) assay (Hologic, Inc., San Diego, CA) and the Roche cobas® TV/MG assay (cobas) (25, 30-32). In 2015, the US Centers for Disease Control and Prevention (CDC) recognized MG infections as an emerging concern and described the need for improvements in diagnosis and treatment of these infections (25). The British Association for Sexual Health and HIV (BASHH) and the International Union Against Sexually Transmitted Infections (IUSTI) both recommend that symptomatic patients should be tested for MG infection using NAAT technologies (3, 33). The objective of this multicenter study was to evaluate the clinical performance of the cobas test for the detection of MG, using urogenital specimens from both symptomatic and asymptomatic men and women.

METHODS

Patient population and ethics

This multicenter study enrolled 2,194 participants aged ≥14 years, who reported sexual activity within the previous 6 months. Participants attending family planning, obstetrics and gynecology, and STI clinics were recruited from geographically diverse sites in the US: Birmingham (AL), Indianapolis (IN), Jackson (MI), Miami (FL), New Haven (CT), New Orleans (LA), Oakland (CA), Providence (RI), and St Louis (MO) (supplemental Figure 1).

Participants were classified as demonstrating signs of infection if they reported any of the following symptoms: dysuria, coital issues (pain, difficulty, or bleeding), pelvic pain, abnormal vaginal discharge, unusual vaginal odor pelvic, uterine or ovarian pain, penile discharge, testicular pain, scrotal pain, or swelling, itching, burning, redness or soreness of the genitals.

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Patients were ineligible if they had previously enrolled in the study; used antimicrobial agents active against MG (doxycycline; macrolides, including azithromycin and erythromycin; or fluoroquinolones, including ofloxacin, ciprofloxacin, levofloxacin) within the 21 days prior to sample collection; used Replens (Church & Dwight, Co., Inc., Princeton, NJ), RepHresh Odor Eliminating Vaginal Gel, RepHresh Clean and Balance (Church & Dwight, Co., Inc., Princeton, NJ) or products containing metronidazole within 3 days prior to specimen collection; had undergone a full hysterectomy; or had a contraindication to the Papanicolaou Test or cervical sampling.

This study was conducted in compliance with the International Conference on Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), Good Clinical Practice Guidelines (GCP), and applicable US Food and Drug Administration (FDA) regulations and all participating subjects provided written informed consent. Institutional Review Board approval was obtained from each participating study site prior to the start of the study.

Specimen collection

Women provided specimens in the following order: a first catch urine (FCU), vaginal swabs, an endocervical swab in cobas PCR media, and a cervical specimen in PreservCyt® Solution obtained with a spatula, cytobrush, or broom. Participants were randomized to either the self-obtained or the clinician-obtained for collection of vaginal swabs used in the cobas assay.

Participants within the self-collected arm had their self-collected vaginal swab collected first, and the remaining swabs were clinician-collected. In the clinician-collected arm, all vaginal swabs were clinician-collected. Following collection, the clinician transferred the swabs to

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the relevant transport media, as per the respective laboratory's standard operating procedures, for the validated APT MG assay (Hologic, San Diego, CA) and two MG laboratory-developed tests (LDTs) (34-36). Participants within the clinician-collected arm had an additional clinician-collected specimen for use with the cobas test. Both the endocervical swab and the liquid-based cytology (LBC) sample were collected for assessment with the cobas assay only.

Men first provided meatal swabs (self or clinician-collected) for use with the cobas test, followed by an FCU sample. The FCU sample was aliquoted into the manufacturer's collection device for use with APT MG, the two other MG LDTs, and the cobas assay.

Sample testing

The cobas assay was tested on either the cobas 6800 or 8800 system (detection of MG with the cobas assay is FDA-cleared for female urine, self- and clinician-collected vaginal swabs, endocervical swabs, male urine, and male meatal swabs only). Specimens from each individual subject were tested using the cobas assay at a single test site. Samples for comparator methods were tested at sites based on the availability of the comparator instrument system and method. Samples were coded to ensure they were anonymized and to reduce bias. Testing was performed with each method according to the the validated laboratory procedure (for the three LDTs). One of the MG LDTs was a real-time PCR assay that targeted the *mgpA* gene of MG (34, 35). The other MG LDT was a quantitative PCR designed to target the 23S rRNA gene of MG (36). The APT MG assay detects the 16S rRNA of MG.

Patient infected status (PIS)

The PIS was determined from vaginal swabs (women) and FCU (men) assayed in two MG laboratory-developed NAATs and the APT MG assay. If a participant had two or more positive results the PIS was 'positive', and at least two negative results defined the 'not infected' classification. Any other combination of valid result with invalid results were considered 'indeterminate'. Performance estimates for all sample types were based on comparison to these PIS classifications.

Data analysis and interpretation of results

Test results for each assay were interpreted according to the testing laboratory's SOP and validation for their respective MG assay. Results were deemed invalid if there were protocol deviations, incidents, or if the data were generated during troubleshooting of the instrument or assays. All data analyses were performed using SAS/STAT® software (37).

The clinical performance of the cobas test for the detection of MG was evaluated by comparing test results to the PIS. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated overall, for each gender, by specimen type and symptom status, and compared with the infected status. The two-sided 95% confidence intervals (CIs) were provided for the estimates of sensitivity, specificity, PPV, and NPV. Significance was defined using Z-test analysis with alpha=0.05.

RESULTS

Subject disposition

Of the 2,194 participants enrolled in the study, a total of 2,154 were considered eligible and 2,150 were evaluated (1,104 female and 1,046 male) for the assessment of MG infection (Table 1). Evaluable urine samples were available from 1,099 female and 1,045 male participants. Clinician-collected and self-collected vaginal swabs were available in 551 and

550 participants, respectively. Clinician-collected and self-collected penile meatal swabs were available from 516 and 522 participants, respectively. In total, 28 specimens were excluded from the analysis: 5 female urine, 2 clinician-collected vaginal swabs, 1 self-collected vaginal swab, 6 PreservCyt, 5 endocervical swabs, 1 male urine, 2 clinician-collected meatal swab, 2 self-collected meatal swabs, and 4 meatal swabs without collection information.

Assay performance for the detection of MG

In total, 59 women and 60 men were considered infected as determined by PIS analysis. Of these infected participants, 67.8% of women and 51.7% of men reported symptoms. The sensitivity, specificity, PPV, and NPV of cobas for the detection of MG are shown in Table 2. The overall sensitivity of the cobas test for the detection of MG in women was highest in vaginal swab samples (96.6% [95% CI 88.5–99.1], clinician- and self-collected combined). The overall sensitivity of the test for female urine, PreservCyt samples and endocervical samples ranged from 83.1% to 86.4% (Table 2). The overall sensitivity of cobas for MG in male urine samples and meatal swab samples was 100% (95% CI 94.0–100%) and 85.0% (95% CI 73.9–91.9), respectively. There were no statistically significant sensitivity differences between the clinician- and self-collected vaginal swabs (96.3% vs 96.9%, respectively, p>0.99) and meatal swabs (83.9% vs 86.2%, respectively, p>0.99) as determined by the Z-test analyses. Additional Z-test analyses similarly showed no statistically significant specificity differences between the clinician- and self-collected vaginal swabs (96.8% vs 97.3%, respectively, p=0.63) and meatal swabs (97.5% vs 98.2%, respectively, p=0.74). Venn diagrams comparing cobas MG positivity across all tests, regardless of PIS, in female urine, male urine, vaginal, and meatal swab samples are shown in Figure 1. The specificity of the cobas assay for MG ranged from 96.0–99.8% across male and female, symptomatic and asymptomatic samples (Table 2).

Based on PIS, MG prevalence was higher in symptomatic than asymtomatic patients and the overall prevalence ranged from 5.4% to 5.8% across male and female specimens (Table 2). The PPV of the cobas for detection of MG was 58.6–94.7%, and the NPV was 98.7–100% across all specimen types evaluated. Additional analyses of MG (regardless of PIS) prevalence by age, gender, sample type, and study site are provided in supplemental Tables 1 and 2.

DISCUSSION

This multicenter study evaluated the clinical performance of the cobas test for the detection of MG in urine, and genital swab samples from men and women. Male urine and female vaginal swab samples had the highest sensitivity and specificity for detection of MG in this analysis. The evidence supporting optimal specimen collection for MG detection in urogenital specimens is evolving. Observed differences among specimen types maybe associated with pathogenesis and anatomical location (38, 39). The prevalence of MG varied among female specimens (Supplemental Table 2). However, the differences between specimen types for men were not significant. The only statistically significant differences among female samples were between cervical (PreservCyt®) and endocervical swabs, which were significantly less sensitive when compared with vaginal swabs (Table 2; p-values <0.0001).

The cobas test for the detection of MG had similar performance when assessed in both self-collected and clinician-collected vaginal or meatal swabs. This is important as self-collection allows patients who are not comfortable with visiting a clinic or clinician collection, access to effective testing. Across the STI testing field, self-testing has provided increased access to testing for patients who otherwise may not have received testing and is considered to have similar performance to testing with clinician-collected samples (40-43).

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Specificity is important to ensure a patient is truly positive for the test infection. This is particularly important when introducing new NAATs to become the standard of care when gold-standard culture tests have historically been unavailable. The specificity of the cobas TV/MG test for the detection of MG was high regardless of the sample type or symptom status (Table 2) indicating the ability to perform well in different patient populations. In the absence of a reliable gold-standard test for detection of MG, the first FDA-approved assay (Hologic Aptima) was validated by comparison to three alternate TMA LDTs (18, 44). Here we provide a similar evidence base for the cobas assay, allowing comparison with three validated LDTs (two PCR and one TMA-based method). Table 3 shows the head-to-head comparisons of cobas with the individual MG LDT NAATs for the US prospective clinical study and highlights the variability that may be observed with different laboratories using validated LDTs for diagnosis of a suspected MG infection.

This prospective clinical study assessed the performance of the cobas assay for detecting MG among both symptomatic and asymptomatic patients. Current European and BASHH guidelines recommend testing of symptomatic individuals, but it is left to the discretion of the healthcare provider whether testing is warranted in those who are asymptomatic. In agreement with this study, the European and BASHH guidelines currently recommend that FCU samples in male participants and female vaginal swabs are the most sensitive sample types (3, 33). This study did not include ano-rectal samples in the evaluation since such studies should be conducted in more specialized clinical settings providing services to men who have sex with men. This is an important area for future assay evaluations.

In this multicenter clinical study, the cobas assay had a high sensitivity and specificity for the detection of MG in both male and female sample types, regardless of symptom status.

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This study provides evidence of a fully validated, high-throughput PCR assay for the detection of MG. Diagnostic solutions that include resistance markers in addition to detection of the organisim may be necessary in the near future. A useful aspect of the cobas 6800/8800 system is that LDTs can be rapidly developed and implemented on this platform, as reflex test options for MG positive specimens are required (45).

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Disclosures

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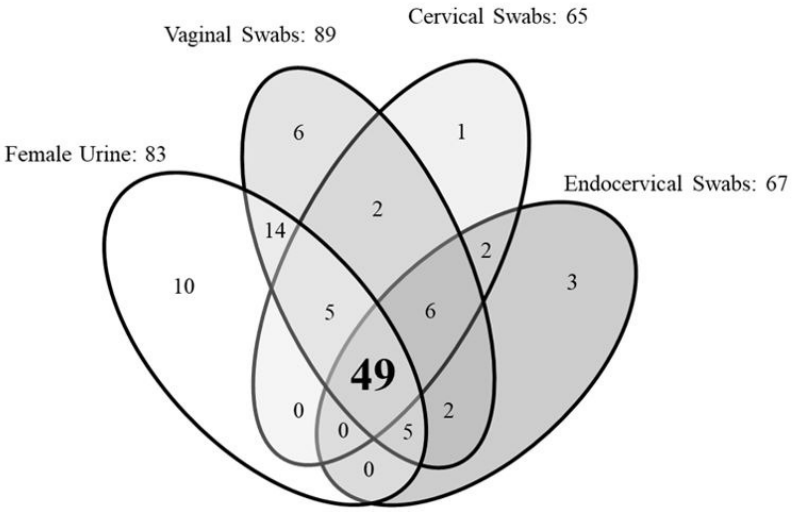
436
437 FIGURE 1. VENN DIAGRAMS COMPARING MG POSITIVE A) FEMALE UROGENITAL SAMPLES
438 AND B) MALE UROGENITAL SAMPLES.
439

These data show exclusively cobas MG positive, results as each sample type was not tested by all comparator assays.

MG, *Mycoplasma genitalium*; PIS, patient infected status.

A



B

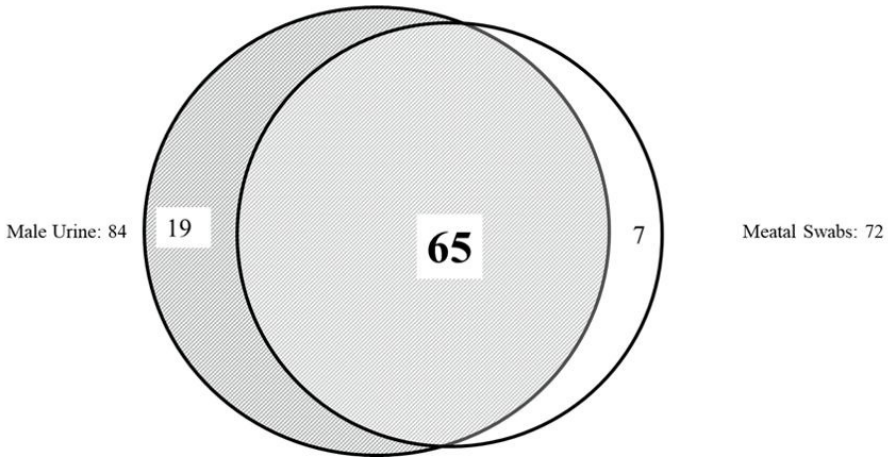


TABLE 1. BASELINE DEMOGRAPHICS AND CHARACTERISTICS

Characteristic	
Total (N)	2150
Male age, years (mean ± SD)	37.6 + 13.6
Female age, years (mean ± SD)	34.2 + 11.7
Male (N [%])	1,046 (48.7%)
Female (N [%])	1,104 (51.3%)
American Indian/Alaskan Native (N [%])	3 (0.1%)
Asian (N [%])	13 (0.6%)
Black/African American (N [%])	1,501 (69.8%)
Native Hawaiian/Pacific Islander (N [%])	5 (0.2%)
White (N [%])	553 (25.7%)
Multiple/Other (N [%])	55 (2.6%)
Not reported (N [%])	20 (0.9%)
Symptomatic (N [%])	984 (45.8%)
Asymptomatic (N [%])	1,166 (54.2%)
Pregnant (female only (N [%]))	3 (0.3%)
Family planning clinic (N [%])	525 (24.4%)
Obstetrics/gynecology clinic (N [%])	273 (12.7%)
STI clinic (N [%])	758 (35.2%)
Family planning/STI clinic (N [%])	594 (27.6%)

SD, standard deviation; STI, sexually transmitted infection.

1 TABLE 2. CLINICAL PERFORMANCE COMPARED WITH PIS BY GENDER, SAMPLE TYPE, AND SYMPTOM STATUS

Sample type	Total	Sensitivity % (N/N)	95% CI	Specificity % (N/N)	95% CI	Prevalence %	PPV %	NPV %
Female participants								
Urine								
Symptomatic	636	85.0 (34/40)	70.9–92.9	96.0 (572/596)	94.1–97.3	6.3	58.6	99.0
Asymptomatic	463	89.5 (17/19)	68.6–97.1	98.4 (437/444)	96.8–99.2	4.1	70.8	99.5
Overall	1099	86.4 (51/59)	75.5–93.0	97.0 (1009/1040)	95.8–97.9	5.4	62.2	99.2
Vaginal swab (both clinician- and self-collected)								
Symptomatic	639	97.5 (39/40)	87.1–99.6	96.3 (577/599)	94.5–97.6	6.3	63.9	99.8
Asymptomatic	462	94.7 (18/19)	75.4–99.1	98.0 (434/443)	96.2–98.9	4.1	66.7	99.8
Overall	1101	96.6 (57/59)	88.5–99.1	97.0 (1011/1042)	95.8–97.9	5.4	64.8	99.8
PreservCyt samples								
Symptomatic	638	80.0 (32/40)	65.2–89.5	97.8 (585/598)	96.3–98.7	6.3	71.1	98.7
Asymptomatic	460	94.7 (18/19)	75.4–99.1	99.8 (440/441)	98.7–100	4.1	94.7	99.8

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Overall	1098	84.7 (50/59)	73.5–91.8	98.7 (1025/1039)	97.8–99.2	5.4	78.1	99.1
Endocervical swab								
Symptomatic	637	85.0 (34/40)	70.9–92.9	97.7 (583/597)	96.1–98.6	6.3	70.8	99.0
Asymptomatic	462	78.9 (15/19)	56.7–91.5	99.3 (440/443)	98.0–99.8	4.1	83.3	99.1
Overall	1,099	83.1 (49/59)	71.5–90.5	98.4 (1023/1040)	97.4–99.0	5.4	74.2	99.0
Male participants								
Urine								
Symptomatic	343	100 (31/31)	89.0–100	96.8 (302/312)	94.2–98.2	9.0	75.6	100
Asymptomatic	702	100 (29/29)	88.3–100	97.9 (659/673)	96.5–98.8	4.1	67.4	100
Overall	1,045	100 (60/60)	94.0–100	97.6 (961/985)	96.4–98.4	5.7	71.4	100
Meatal swab (both clinician- and self-collected)								
Symptomatic	343	90.3 (28/31)	75.1–96.7	96.5 (301/312)	93.8–98.0	9.0	71.8	99.0
Asymptomatic	695	79.3 (23/29)	61.6–90.2	98.5 (656/666)	97.3–99.2	4.2	69.7	99.1
Overall	1,038	85 (51/60)	73.9–91.9	97.9 (957/978)	96.7–98.6	5.8	70.8	99.1

2 CI, confidence interval; NPV, negative predictive value; PIS, patient infected status; PPV, positive predictive value.

TABLE 3. THE AGREEMENT OF THE COBAS FOR MG WITH EACH NAAT

A. VAGINAL SWABS

cobas	NAAT1[a] MG positive	NAAT1 MG negative	Total	NAAT2[b] MG positive	NAAT2 MG negative	Total	NAAT3[c] MG positive	NAAT3 MG negative	Total
MG positive	36	52	88	55	33	88	88	0	88
MG negative	13	999	1,012	10	1,002	1,012	26	986	1,012
Total	49	1,051	1,100	65	1,035	1,100	114	986	1,100
PPA (95% CI)	73.5 (59.7–83.8)%			84.6 (73.9–91.4)%			77.2 (68.7–83.9)%		
NPA (95% CI)	95.1 (93.6–96.2)%			96.8 (95.6–97.7)%			100 (99.6–100)%		
OPA (95% CI)	94.1 (92.5–95.3)%			96.1 (94.8–97.1)%			97.6 (96.6–98.4)%		

B. MALE URINE SAMPLES

cobas	NAAT1[a] MG positive	NAAT1 MG negative	Total	NAAT2[b] MG positive	NAAT2 MG negative	Total	NAAT3[c] MG positive	NAAT3 MG negative	Total
MG positive	57	27	84	52	32	84	79	5	84
MG negative	12	943	955	5	950	955	3	952	955

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Total	69	970	1,039	57	982	1,039	82	957	1,039
PPA (95% CI)	82.6 (72.0–89.8)%			91.2 (81.1–96.2)%			96.3 (89.8–98.7)%		
NPA (95% CI)	97.2 (96.0–98.1)%			96.7 (95.4–97.7)%			99.5 (98.8–99.8)%		
OPA (95% CI)	96.2 (94.9–97.2)%			96.4 (95.1–97.4)%			99.2 (98.5–99.6)%		

[a]NAAT1 = LDT 1 (targets mgbA gene), [b]NAAT2 = LDT 2 (targets 23S rRNA), [c]NAAT3 = LDT3 (targets 16S rRNA)

CI, confidence interval; cobas, cobas TV/MG; LDT, laboratory-developed test; MG, *Mycoplasma genitalium*; NAAT, nucleic acid amplification test; NPA, negative percentage agreement; PPA, positive percentage agreement; OPA, overall percentage agreement.