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Quality control for diagnostic oral microbiology laboratories in European countries

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Participation in diagnostic microbiology internal and external quality control (QC) processes is good laboratory practice and an essential component of a quality management system. However, no QC scheme for diagnostic oral microbiology existed until 2009 when the Clinical Oral Microbiology (COMB) Network was created. At the European Oral Microbiology Workshop in 2008, 12 laboratories processing clinical oral microbiological samples were identified. All these were recruited to participate into the study and six laboratories from six European countries completed both the online survey and the first QC round. Three additional laboratories participated in the second round. Based on the survey, European oral microbiology laboratories process a significant (mean per laboratory 4,135) number of diagnostic samples from the oral cavity annually. A majority of the laboratories did not participate in any internal or external QC programme and nearly half of the laboratories did not have standard operating procedures for the tests they performed. In both QC rounds, there was a large variation in the results, interpretation and reporting of antibiotic susceptibility testing among the laboratories. In conclusion, the results of this study demonstrate the need for harmonisation of laboratory processing methods and interpretation of results for oral microbiology specimens. The QC rounds highlighted the value of external QC in evaluating the efficacy and safety of processes, materials and methods used in the laboratory. The use of standardised methods is also a prerequisite for multi-centre epidemiological studies that can provide important information on emerging microbes and trends in anti-microbial susceptibility for empirical prescribing in oro-facial infections.

Keywords: *diagnostics*; *multicentre*; *oral microbiology*; *periodontitis*; *endodontic*; *periodontal pocket*; *root canal*; *quality control*

Infections of the oral mucosa, teeth (caries and root canal infections) and their supporting structures (periodontitis, dento-alveolar abscess) are polymicrobial, although usually associated with a characteristic microbiota linked to the site of infection (1). However, identification of the relevant oral pathogens is not commonly undertaken in diagnostic clinical microbiology laboratories due to lack of expertise in handling fastidious oral microbes and interpretation of the findings. When specimens from oral diseases are processed, they are frequently reported as 'mixed oral flora'. This type of reporting is unhelpful both to clinicians and to epidemiologists collating data for disease and anti-microbial susceptibility trends. This probably reflects the lack of input by experts in oral microbiology into general guidelines for processing and reporting samples from the oral cavity. Although there is a general consensus within the dental and maxillo-facial surgery community on the role of the major pathogens for many types of infection, there is controversy on the role of some species, first line

anti-microbial agents and their associated breakpoints. Furthermore, there is poor uptake and understanding of microbiology diagnostic services by the dental profession, inappropriate specimens and misinterpretation of culture results (2, 3).

Microbiological diagnostics relies on the quality of the test performance. This can be assured by internal controls: testing the ability to culture, identify and determine the anti-microbial profiles for indicator micro-organisms with known susceptibility profiles. Standard operating procedures (SOPs) and internal audits further improve the accuracy and quality of the laboratory performance. However, such data must be laboratory independent, and exposure to external audits and quality control (QC) rounds is essential. There are proficiency schemes available for clinical microbiology laboratories such as the United Kingdom National External Quality Assessment Service (UK NEQAS; http://www.ukneqas-micro.org.uk) and the Quality Control for Molecular Diagnostics (QCMD; http://www.qcmd.org) for general microbiology. However, external QC schemes for diagnostic oral microbiology are not available in any of the European countries. To fulfil the requirements of diagnostic laboratory accreditation, the adoption of an external QC process is mandatory in many countries. In the 2008 European tri-annual meeting of oral microbiology specialists (European Oral Microbiology Workshop 2008, Helsinki, Finland), a number of diagnostic oral microbiology laboratories agreed to participate in the collation of data from diagnostic laboratory processes, interpretative criteria and quality assurance. The Clinical Oral Microbiology (COMB) Network was created to provide external QC rounds for member laboratories and to collaborate on SOPs for processing and reporting samples from the oral cavity.

The aim of this study was to evaluate the range of oral infection specimens processed, diagnostic methods, internal and external QC programmes used in European diagnostic oral microbiology laboratories by an online questionnaire. In addition, we determined the performance of respondent European oral microbiology diagnostic laboratories in processing and reporting blinded spiked oral specimens from hypothetical oral disease scenarios.

Materials and methods
At the European Oral Microbiology Workshop 2008 (EOMW2008), Helsinki, Finland, 12 laboratories processing clinical oral microbiological samples were identified. All these were recruited to participate in the study and seven originating from six European countries completed both the online survey and the first QC round. Three additional laboratories participated in the second QC round.

Survey
The online survey consisted of a total of 18 open and closed questions on the numbers and types of specimens processed, molecular methods used, SOPs and participation in internal and external QC. The link to the survey was sent to representatives of all 12 laboratories identified at the EOMW 2008 meeting.

First QC round
A periodontal pocket QC specimen was prepared by spiking sub-gingival plaque from a healthy volunteer with the following pathogens: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Parvimonas micra*. The QC specimen (approximately 10^{10} cfu/mL) was prepared in thioglycolate broth of which 100 μl was transferred into anaerobically prepared 2 ml VMGAIII transport medium vials (4) and sent to participating laboratories by a courier. The specimen was accompanied with the following information: 'The specimen is from a 7 mm periodontal pocket, upper 1st molar in a 56-year old male patient with type II diabetes and is allergic to penicillin'. Participants were asked to analyse the specimens using their normal SOPs and methods. Laboratories were also asked to perform antibiotic susceptibility tests on significant isolates. Information on the assays, culture techniques and media used, as well as transportation time, were recorded in a standardised data collection sheet. A copy of a standard report was also collated. The laboratory providing the round cultured a control sample after 3 days of storage at room temperature.

Second QC round
An endodontic QC specimen was prepared into Luria-Bertani broth, Lennox (BD Difco, Sparks, MD, USA) by spiking with an *Escherichia coli* (NCTC 13353; extended spectrum beta-lactamase, extended spectrum beta-lactamase (ESBL) positive), alpha haemolytic streptococci (non-speciated) and *Fusobacterium nucleatum* (final concentration approximately 10^{10} cfu/mL). Three paper points (#50) were soaked in the spiked broth and transferred into VMGAIII transport medium vial (4) and sent to participating laboratories by a courier. The specimen was accompanied with the following information: 'Root treatment started four days ago (old filling fell out one month ago), now a flare up, lots of pus from root canals. Patient has chronic obstructive pulmonary disease (COPD) and is allergic to penicillin'. The laboratories were asked to analyse and report the specimens as previously. The laboratory providing the round cultured a control sample after 3 days of storage at room temperature.

Results

Survey

Based on the survey questionnaire, each laboratory processed, on average, a total of 4,135 samples per annum. Five of the seven laboratories (71%) did not participate in any internal or external QC programme, and three (43%) of the participating laboratories did not have SOPs for the tests they performed. The numbers and types of samples processed by the participant laboratories are summarised in Fig. 1. The most common sample type processed was sub-gingival plaque from patients with various forms of periodontal disease: mean 3,037 samples/laboratory annually (range 10–16,000). All laboratories processed these samples. Other clinical samples processed were mucosal swabs, pus swabs and aspirates (from dento-alveolar infections) in addition to performing dental caries susceptibility tests using stimulated saliva as a clinical specimen. However, the mean annual numbers for these were low: mean 118, 68, 77 and 134 samples/laboratory annually, respectively (range 0–400).

First QC round

Six laboratories processed, analysed and reported the sample within the specified time of 3 weeks. The mean transportation time was 1.8 (1–3) days. The methods used for anaerobic culture included conventional or fastidious anaerobe blood agar (usually Columbia agar base supplemented with defibrinated horse or sheep blood (5%), haemin (5 mg/l) and menadione (1–10 mg/l)), and on selective media, such as tryptic soy serum bacitracin vancomycin agar (TSBV) for the cultivation of *A. actinomycetemcomitans* (5). Two laboratories used PCR techniques. One laboratory used a Real-Time PCR-panel for periodontal pathogens (6, 7). Another laboratory used conventional PCR with agarose gel electrophoresis (8).

The findings reported by the laboratories are listed in Table 1. Six laboratories reported one or more periodontal pathogens from the sample. The predominant pathogens (*A. actinomycetemcomitans, P. gingivalis*) were identified by three of six laboratories. The most commonly identified finding was *A. actinomycetemcomitans* (five of six laboratories). *P. gingivalis* and *P. micra* were recovered by three of six laboratories. One laboratory reported that periodontal pathogens were not found. Three laboratories identified and reported a number of oral streptococci. The laboratory providing the round detected all spiked pathogens.

Five laboratories performed susceptibility testing primarily by disc diffusion. There was large variation in the antibiotic susceptibility testing performed. Most often susceptibility testing was performed for *A. actinomycetemcomitans* ($n = 4$) (Table 2). It was reported susceptible to penicillin (two of three), amoxicillin (three of three) and doxycycline/tetracycline (three of four) and resistant
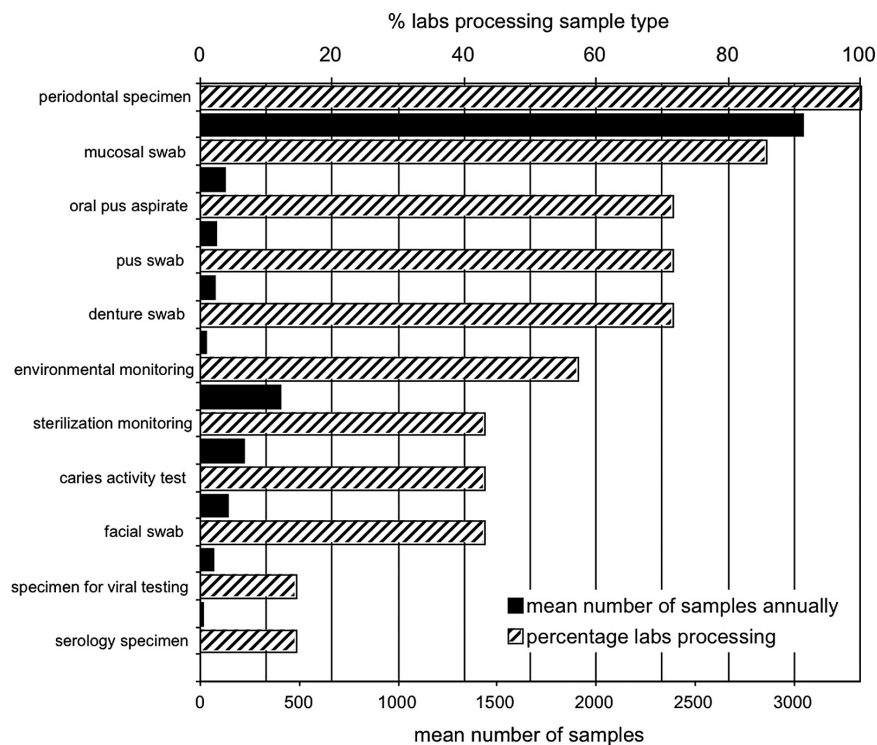

Fig. 1. Proportion of participant laboratories ($n = 6$) that processed the indicated samples (hatched bars and upper *x*-axis) on a routine basis and annual mean number of samples processed by the laboratories (solid bars and lower *x*-axis).

Table 1. Isolates recovered by culture techniques and species identified by PCR from the Round 1 sub-gingival plaque sample spiked with *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Parvimonas micra* analysed by six independent laboratories from six European countries

Laboratory	Culture techniques						PCR	
	1	2	3	4	5	6	1	4
Isolate	Percentage of total flora			Semi quantitative amount			PCR signal	
Streptococcus agalactiae					+			
Streptococcus sanguis					+			
Streptococcus sp.				+		+		
Abiotrpohia adjacens					+			
Actinomyces odontolyticus				+				
Aggregatibacter actinomycetemcomitans	0.5	2.0	1.6	+	+	−	+	+
Porphyromonas gingivalis	80.9	10.0	31.0			−	+	+
Porphyromonas sp.				+				
Parvimonas micra	4.1	7.0	0.5				+	
Tannerella forsythia	0.7	0	0				+	−
Fusobacterium nucleatum	0.15						+	
Fusobacterium sp.				+				
Prevotella intermedia/nigrescens	0	<0.01	0				−	−
Prevotella melaninogenica				+				
Prevotella sp.						+		
Veillonella sp.				+				
Total bacterial count (cfu/ml)	5.5×10^7	1×10^8	1.1×10^7					
Transportation time	1 day	2 days	3 days	1 day	1 day	1 day		

to clindamycin (three of three) and erythromycin (three of three I+R). One laboratory reported that the isolate was susceptible to metronidazole and two laboratories reported that the isolate was resistant to metronidazole.

Second QC round
Nine laboratories processed and reported the sample within the specified time of 3 weeks. The mean transportation time was 3.6 (2–6) days. All nine laboratories had identified a heavy growth of a coliform. Six of these identified the coliform as *Escherichia coli* and five identified it as an ESBL producer. In addition, one laboratory which did not name the coliform to species level identified it as an ESBL producer. The *E. coli* was identified by VITEK (*n* = 3) or by biochemical tests (one by API 20E and one using API 32E (Biomerieux, La Balme Les Grottes, France) and one not specified) (*n* = 3). One laboratory reported that VITEK 2 had flagged the *E. coli* as O157 requiring referral to a reference laboratory, and no further processing of the sample was carried out due to infection control guidance. Two of the six laboratories that identified the ESBL-producing *E. coli* highlighted the need to implement infection control precautions in their report. Four laboratories isolated and identified the alpha haemolytic streptococci to species level and three of these reported anti-microbial susceptibility data on this isolate. One laboratory reported the isolate resistant to penicillin by disc diffusion. None of the laboratories recovered and identified *Fusobacterium nucleatum*. The laboratory providing the round detected all spiked pathogens.

Table 2. Antibiotograms reported for *A. actinomycetemcomitans* by four participant laboratories (S, susceptible; I, intermediate; R, resistant)

AB LAB	Pen	Amox	Ery	Clinda	Doxy/tet	Met	Amox-Cla
1		S			S	S	
2	S	S	I	R	S	R	S
4	R		R	R	S	R	
5	S	S	I	R	R		

Discussion
Participation in diagnostic microbiology internal and external QC processes is good laboratory practice and an essential component of laboratory quality management system. In many European countries, it is compulsory for all diagnostic laboratories to participate. However, no QC scheme for diagnostic oral microbiology

existed until 2009 when the COMB Network was created. The results of this study show that European microbiology laboratories process a significant number of diagnostic samples from the oral cavity annually. At the same time, the majority of the surveyed laboratories did not participate in any internal or external QC programme and nearly half of the laboratories did not have SOPs for the tests they performed.

Laboratories reported that they all processed periodontitis samples routinely. Therefore, it was decided to provide a periodontitis QC specimen in the first instance. Most of the laboratories were able to recover the microaerophilic *A. actinomycetemcomitans* by culture. However, only half of the laboratories recovered and identified the anaerobic pathogens in the specimen. The two PCR methods used by the laboratories in this study appeared to be equally sensitive in detecting the pathogens. Interestingly, *Tannerella forsythia* was detected by one of the PCR methods. The sample was not spiked with this pathogen, but it is possible that it was present in low numbers in the sub-gingival plaque from the healthy volunteer used in preparation of the sample. Therefore, it is possible that not all samples had detectable amounts of *T. forsythia*. Other laboratories identified and reported a number of facultative commensals of dental plaque as pathogens reflecting lack of consensus regarding the causality of the disease. These results highlighted the challenges of recovering and identifying anaerobic bacteria by culture methods.

The results of the second round endodontic QC specimen from a COPD patient also highlighted the challenges of anaerobe diagnostics because no laboratory was able to recover the *F. nucleatum* from the sample. A high inoculum of *E. coli* may have inhibited or masked the growth of *F. nucleatum*. All nine laboratories reported heavy growth of a coliform, six of which identified it as an ESBL producer. However, only two of these laboratories highlighted the need to take infection control precautions in their report. Many hospitals make every effort to isolate patients colonised with resistant pathogens, although guidelines vary nationally. Both laboratories that mentioned the need for infection control measures were attached to larger general microbiology laboratories. Updating and maintaining quality is a major challenge for small isolated laboratories. Open access guidelines and SOPs for processing and reporting oral samples would provide minimum level of standardisation. This QC highlighted the importance of knowledge of current anti-microbial susceptibility mechanisms linked to infection prevention issues.

In both QC rounds, there was large variation in the results, interpretation and reporting of antibiotic susceptibility testing among the laboratories. This is not surprising considering the various methodologies and media used. In addition, there are no clinically proven break points for a number of oral pathogens, and interpretation is frequently based on the literature from structurally similar pathogens resulting in discordant results. This highlights the importance of closer cooperation between diagnostic oral microbiology laboratories within Europe. Considering the large number of patients with oro-facial infections, it is disappointing that use of diagnostic microbiology facilities is not utilised to a greater extent. One of the challenges in working in the speciality of diagnostic oral microbiology is the relatively small numbers of laboratories undertaking such work in Europe. This QC has demonstrated the importance of closer collaborative working not just only for SOPs and reporting but also for training and developing a concensus in clinical interpretation of results and communication to clinicians and other medical microbiology colleagues.

In conclusion, the results of this study demonstrate the need for harmonisation of laboratory processing methods and interpretation of results for oral microbiology specimens. The use of standardised methods is also a prerequisite for multi-centre epidemiological studies that can provide important information on emerging microbes and trends in anti-microbial susceptibility for empirical prescribing in oro-facial infections. The results of the QC rounds also highlighted the value of external QC in evaluating the efficacy and safety of processes, materials and methods used in the laboratory. Historically, anaerobic culture methods are recognised as the gold standard and molecular methods as a second line alternative (9–11). The results of these QC rounds should trigger a critical evaluation of the sensitivity and reliability of the culture methods used for the detection of anaerobic oral bacteria. This exercise has also fostered collaborations between a network of reference laboratories in the EU identifying and characterising relevant pathogens from oral infections, this should also provide a useful resource for diagnostic medical microbiology laboratories.

Conflict of interest and funding

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References

1. Peterson LR, Thomson RB, Jr. Use of the clinical microbiology laboratory for the diagnosis and management of infectious diseases related to the oral cavity. Infect Dis Clin North Am 1999; 13: 775–95.
2. Roy KM, Smith A, Sanderson J, Bagg J, MacKenzie D, Jackson MS, et al. Barriers to the use of a diagnostic oral microbiology laboratory by general dental practitioners. Br Dent J 1999; 186: 345–7.
3. Palmer NO, Martin MV, Pealing R, Ireland RS, Roy K, Smith A, et al. Antibiotic prescribing knowledge of national health service general dental practitioners in England and Scotland. J Antimicrob Chemother 2001; 47: 233–7.
4. Van Steenbergen TJ, Petit MD, Tijhof CJ, Van Winkelhoff AJ, Van der Velden U, De Graaff J. Survival in transport media of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* in human subgingival samples. Oral Microbiol Immunol 1993; 8: 370–4.
5. Slots J. Selective medium for isolation of *Actinobacillus actinomycetemcomitans*. J Clin Microbiol 1982; 15: 606–9.
6. Kuboniwa M, Amano A, Kimura KR, Sekine S, Kato S, Yamamoto Y, et al. Quantitative detection of periodontal pathogens using real-time polymerase chain reaction with TaqMan probes. Oral Microbiol Immunol 2004; 19: 168–76.
7. Boutaga K, Van Winkelhoff AJ, Vandenbroucke-Grauls CM, Savelkoul PH. Periodontal pathogens: a quantitative comparison of anaerobic culture and real-time PCR. FEMS Immunol Med Microbiol 2005; 45: 191–9.
8. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol 1996; 11: 266–73.
9. Paju S, Pussinen PJ, Suominen-Taipale L, Hyvonen M, Knuuttila M, Könönen E. Detection of multiple pathogenic species in saliva is associated with periodontal infection in adults. J Clin Microbiol 2009; 47: 235–8.
10. Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ. Prevalence of *Porphyromonas gingivalis* and periodontal health status. J Clin Microbiol 1998; 36: 3239–42.
11. Riggio MP, Macfarlane TW, Mackenzie D, Lennon A, Smith AJ, Kinane D. Comparison of polymerase chain reaction and culture methods for detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in subgingival plaque samples. J Periodontal Res 1996; 31: 496–501.

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