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Journal of Microbiological Methods 61 (2005) 105–126
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

# Comparison of image analysis software packages in the assessment of adhesion of microorganisms to mucosal epithelium using confocal laser scanning microscopy

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Received 9 June 2004; received in revised form 9 November 2004; accepted 19 November 2004
Available online 24 December 2004

**Abstract**

We have compared current image analysis software packages in order to find the most useful one for assessing microbial adhesion and inhibition of adhesion to tissue sections. We have used organisms of different sizes, the bacterium *Helicobacter pylori* and the yeast *Candida albicans*. Adhesion of FITC-labelled *H. pylori* and *C. albicans* was assessed by confocal microscopy. Four different Image analysis software packages, NIH-Image, IP Lab, Image Pro+, and Metamorph, were compared for their ability to quantify adhesion of the two organisms and several quantification methods were devised for each package. For both organisms, the dynamic range that could be detected by the software packages was $1\times10^6$–$1\times10^9$ cells/ml. Of the four software packages tested, our results showed that Metamorph software, using our 'Region of Interest' method, with the software's 'Standard Area Method' of counting, was the most suitable for quantifying adhesion of both organisms because of its unique ability to separate clumps of microbial cells. Moreover, fewer steps were required. By pre-incubating *H. pylori* with the glycoconjugate Lewis b-HSA, an inhibition of binding of 48.8% was achieved using 250 μg/ml Lewis b-HSA. The method we have devised using Metamorph software, provides a simple, quick and accurate way of quantifying adhesion and inhibition of adhesion of microbial cells to the epithelial surface of tissue sections. The method can be applied to organisms ranging in size from small bacteria to larger yeast cells.


*Keywords:* Adhesion; *Candida albicans*; *Helicobacter pylori*; Image analysis

## 1. Introduction

Some superficial mucosal infections may become chronic leading to a persistent acute inflammatory reaction and in some cases persistent symptoms for

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doi:10.1016/j.mimet.2004.11.020

the patient. In the case of infection with *Helicobacter pylori*, the host is unable to eradicate the organism, despite a florid neutrophil response. In the case of recurrent *Candida vaginitis*, the host is similarly unable to eradicate the organism from the mucosa. In both cases, the effectiveness of current anti-microbial therapy is being compromised by an increasing level of resistance of both *H. pylori* and *Candida albicans* to the appropriate agents. It is with this in mind that alternative strategies are being investigated and one such strategy is to target adhesion, the primary step in the infection process (Basset et al., 2003). Microbes frequently adhere to tissue by specific ligand–receptor interactions and once adherent lead to tissue damage and a subsequent host response.

*H. pylori* colonises the stomach of over half the human population. Although most infected people are asymptomatic, in 5–20% of those infected, severe gastroduodenal diseases, including ulcers of the stomach and duodenum, gastric lymphomas, and adenocarcinomas, may occur (Montecucco and Rappuoli, 2001). A number of putative adhesin–receptor interactions have been proposed for *H. pylori* (Evans and Evans, 2000). The best described interaction is that between the BabA outer membrane protein found on the surface of the bacterium and the Lewis b blood group antigen which is expressed by gastric epithelial cells. Borén et al. (1993) used fluorescently labelled *H. pylori* cells and showed localised adherence to the epithelium of stomach sections by fluorescence microscopy. They were able to inhibit adhesion of *H. pylori* to stomach expressing the Lewis b antigen (Lewis b stomach) by first pre-incubating bacteria with Lewis b neoglycoconjugates (analogues of the receptor) before adding the bacteria to the tissue sections. Adhesion and inhibition were quantified by manually counting the number of bacteria bound to the epithelial surface. Other studies have confirmed this (Borén et al., 1994; Ilver et al., 1998).

*C. albicans*, a yeast, is responsible for a wide range of diseases including vaginal infections. Approximately three-quarters of all women will suffer from vaginal candidiasis (VC) at least once in their lifetime and up to 25% of these women will experience recurrent disease (recurrent vaginal candidiasis, RVC), which is distressing and difficult to treat (Mardh et al., 2002). Adhesins that have been studied include Hwp1 (Sundstrom, 2002), mannoproteins, which bind to fucose or *N*-acetylglucosamine (GlcNAc) glycosides on human buccal and vaginal epithelial cells (Calderone, 1993; Critchley and Douglas, 1987a,b) and secretory aspartyl proteinases (Watts et al., 1998). The secretory aspartyl proteinase SAP2 has been found to be important in vaginal infection in a rat vaginitis model (De Bernardis et al., 1999) and SAPs1–3 have generally been shown to be important in mucosal adherence (Monod and Borg-von Zepelin, 2002).

As microbial adhesion is the primary step in the pathogenic process, it has received considerable attention because of the possibility of inhibiting this first interaction and thus preventing infection. The process of adhesion, usually occurring in the presence of hydrodynamic and shear forces, which tend to limit the deposition of microorganisms, has been mathematically analysed in both flow and static systems (Sjollema et al., 1989). Several studies have been performed with a parallel plate device designed to mimic hydrodynamic forces (van Kooten et al., 1992; Millsap et al., 1999; Gomez-Suarez et al., 2001), although much work has been performed in the absence of these forces.

Many methods for studying bacterial adhesion have been used (An and Friedman, 1997), which include direct microscopic counts after suitable staining, flow cytometry, and image analysis of fluorescent labelled organisms (Clyne et al., 1997; Grivet et al., 1999), haemagglutination (Goldhar, 1995), attachment to immobilised molecules on thin-layer chromatography plates (Saitoh et al., 1991), radiolabelling (Mackowiak and Marling-Cason, 1984), ATP quantification (Robrish et al., 1977), and immunological methods (Ofek, 1995). Although valuable, these studies are artificial as they do not mimic tissue very closely and the cells may not be representative of those occurring in the target tissue. A number of studies have investigated adhesion to whole tissue sections (Borén et al., 1993; Falk et al., 1993; Reinhard et al., 2000) and although not ideal (due to the processing of the tissue), they are likely to be more representative of the natural microbial–host interaction.

A fundamental requirement in determining adhesion and inhibition of adhesion is quantification of