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1 INTENDED USE

IDEIA™ *PCE* Chlamydia is an immunoassay utilising dual amplification technology for the detection of Chlamydia antigen in urethral and endocervical swabs and male urine.

2 SUMMARY

The genus Chlamydiae contains three species known to cause infection in humans, *Chlamydia trachomatis*, *Chlamydia psittaci* and *Chlamydia pneumoniae* (TWAR). These organisms are parasitic bacteria with antigenic components closely related to other Gram negative bacteria.

Chlamydia replication is complex involving two distinct forms, the infectious elementary body (EB) and the reproductive reticulate body. Infection is initiated after adhesion to and entry of an EB to a host cell by pinocytosis. Within the vacuole (inclusion) formed by the host cell the EB enlarges and transforms into a reticulate body. A few hours after infection reticulate bodies begin to reproduce rapidly by binary fission utilising the host cell energy supply. Between 24 and 72 hours reticulate bodies commence the transformation to elementary bodies within the inclusion. The reproductive cycle, which lasts between 48 - 72 hours, terminates when the expanding inclusion causes cell disruption which may release approximately 10⁴ elementary bodies to continue the infection process.

Chlamydia trachomatis is one of the most prevalent pathogens in the world causing a variety of human infections. Although it is the commonest cause of sexually transmitted disease^{1,2}, a substantial number of cases are asymptomatic. Complications associated with reproductive tract infections caused by *C. trachomatis* include pelvic inflammatory disease, ectopic pregnancy and infertility in women and epididymitis in men. *C. trachomatis* can also cause acute or sub-acute follicular conjunctivitis which may progress to punctate keratitis, scarring and trachoma. Frequently these symptoms develop in patients with unrecognised genital infections^{1,3}. *Chlamydia trachomatis* ophthalmia neonatorum is a complication in infants born to infected mothers.

Chlamydia pneumoniae and *Chlamydia psittaci* are associated with a range of respiratory tract infections some of which may lead to pneumonia.

Diagnostic methods for detection of Chlamydia include traditional culture techniques which are dependent upon the presence of viable elementary bodies in clinical specimens. Elementary bodies undergo replication in cell culture and the inclusions formed are detected microscopically using conventional or immunofluorescence staining techniques. Most commonly, diagnosis of Chlamydia infection is performed either by direct detection of Chlamydia elementary bodies in clinical specimens using immunofluorescent monoclonal antibody reagents (eg IMAGEN™ Chlamydia) or by detection of Chlamydia antigen by immunoassays⁴. Other methods include techniques such as the polymerase chain reaction for detection of Chlamydia nucleic acids⁵.

Culture techniques are expensive and require specialised laboratory facilities. Methods for detection of nucleic acids also require specialised equipment and laboratory facilities to minimise specificity problems. Direct detection by immunofluorescence staining becomes labour intensive when used to routinely screen large numbers of specimens. Amplified Immunoassay techniques for antigen detection (eg IDEIA™ *PCE* Chlamydia) provide a sensitive, specific and economical means of routinely diagnosing Chlamydial infections^{6,7,8}. IDEIA™ *PCE* Chlamydia uses amplification technology at labelling and signal generation stages in an immunoassay format. The solid phase is coated with a Chlamydia genus specific monoclonal antibody of established specificity. Amplification at the labelling stage is achieved by the use of polymer conjugation technology which results in attachment, at each antigen binding site, of a polymer complex carrying a high ratio of enzyme molecules.* Further amplification is achieved during the signal generation stage using a ready-to-use formulation of DAKO's patented enzyme amplification technology⁴. The presence of Chlamydia antigen in clinical specimens is indicated by a coloured end-point.

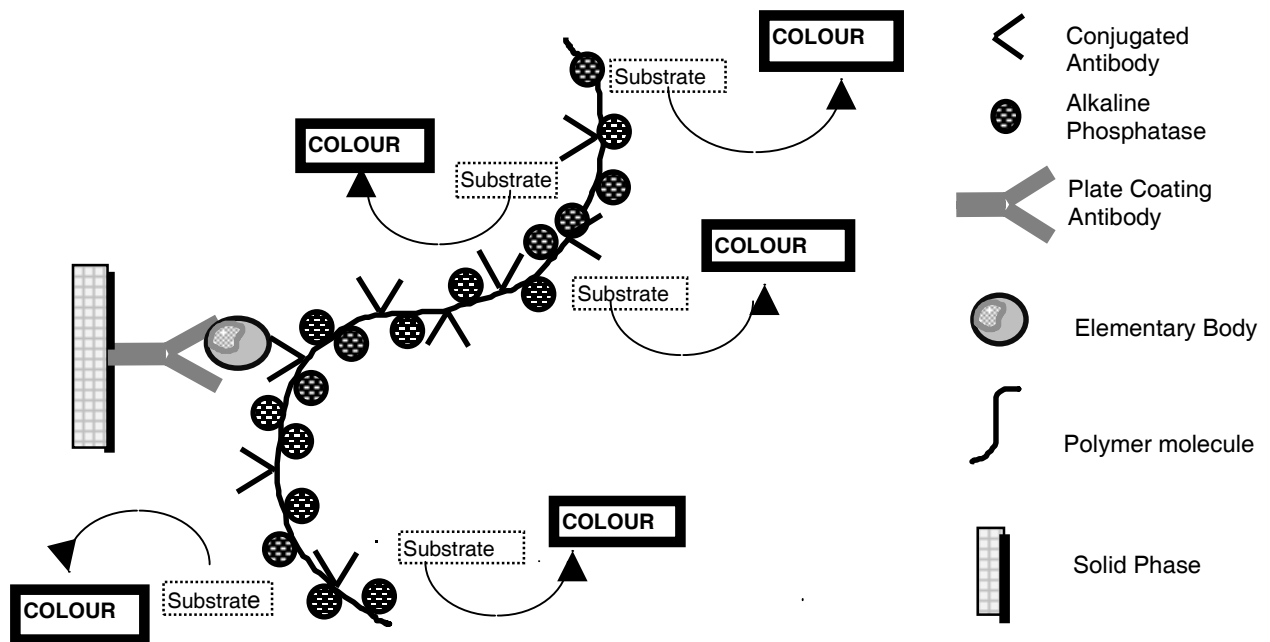
* US Patent No. 5,543,332

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3 PRINCIPLE OF THE TEST

The IDEIA™ *PCE* Chlamydia test utilises a genus specific monoclonal antibody, a polymer conjugate with a high enzyme to antibody ratio³ and a liquid, ready to use enzyme amplification system⁴. Chlamydia antigen, present in human urethral swabs, endocervical swabs and urine (male) specimens, is bound by monoclonal antibody adsorbed to the surface of the solid phase. Conjugate monoclonal antibody binds to Chlamydia antigen captured on the solid phase, thereby linking the conjugate polymer complex carrying multiple enzyme molecules. Washing removes free conjugate complexes. Subsequently, the specifically bound enzyme molecules convert substrate to a colourless product which catalyses the secondary, enzyme signal amplification reaction to produce a coloured endpoint.

Schematic diagram of the IDEIA™ *PCE* Chlamydia assay principle



ACKNOWLEDGEMENT

The monoclonal antibody was produced in the Department of Pathology, Cambridge University, Cambridge, United Kingdom and the Division of Sexually Transmitted Diseases, Clinical Research Centre, Harrow, Middlesex, United Kingdom.

4 REAGENTS PROVIDED

Each kit contains sufficient materials for 192 determinations. The shelf life of the kit is as indicated on the outer box label.

4.1 IDEIA™ *PCE* CHLAMYDIA TEST CONTENTS

One product information booklet.

Two 96 well microtitration plates of twelve, 8-well break-apart strips coated with Chlamydia specific (anti-lipopolsaccharide) monoclonal antibody. A resealable plastic pouch is provided for storage of unused wells.

One bottle of each of the following unless indicated otherwise:

Reagent 1 25mL transport medium concentrate (x10): nonionic detergent in a buffer containing coloured dye, antimicrobial agent and an antifoaming agent.

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Reagent 2

7mL positive control: heat inactivated Chlamydia antigen in buffer solution containing formalin, antimicrobial agent and coloured dye. (Antigen level equivalent to 10^4 - 10^5 elementary bodies per mL).

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Reagent 3	12mL negative control: buffer solution containing antimicrobial agent, coloured dye and an anti-foaming agent.
Reagent 4	2 x 7mL polymer conjugate: Chlamydia specific (anti-lipopolysaccharide) monoclonal antibody conjugated to a dextran polymer backbone linked to multiple alkaline phosphatase molecules in stabilising buffer containing coloured dye and an antimicrobial agent.
Reagent ♦	2 x 125mL washing buffer concentrate (x10): tris buffered solution containing detergent and an antimicrobial agent.
Reagent A	2 x 13mL amplifier A: inorganic salts and buffered enzyme solution containing tetrazolium violet and an antimicrobial agent.
Reagent B	2 x 13mL amplifier B: stabilised NADPH solution.
Reagent X	2 x 13mL stopping solution: 1 mol/L phosphoric acid.

4.2 PREPARATION, STORAGE AND RE-USE OF KIT COMPONENTS

The IDEIA™ *PCE* Chlamydia kit provides for testing of up to twenty four batches of specimens. In order to ensure optimal kit performance, it is important that all unused kit components are stored according to the following instructions.

4.2.1 Monoclonal antibody coated microtitration wells

Open the plate pouch by cutting along the seal. If necessary remove wells from the frame, break off the required number of wells and relocate them into the frame. Place unused wells in the resealable plastic pouch with the dessicant, carefully reseal the pouch and store at 2-8°C. Wells may be used for up to 6 weeks after initial opening, provided they are stored in this manner.

4.2.2 Transport medium concentrate (Reagent 1)

It is important to thoroughly mix the concentrated transport medium prior to diluting it to working strength.

An anti-foaming agent in Reagent 1 causes concentrated and working strength transport medium to appear cloudy which does not affect the test and is not due to microbial contamination.

Prepare working strength transport medium by adding 1 part of Reagent 1 to 9 parts of fresh deionised or distilled water. Dispense 1mL aliquots of working strength transport medium into clean, heat resistant (100°C) screw-capped vials. Vials of working strength transport medium can be used for specimen collection for up to 12 months if kept at room temperature (15-30°C).

4.2.3 Positive control (Reagent 2)

Ready to use. **Do not heat treat.** Store unused positive control at 2-8°C.

4.2.4 Negative Control (Reagent 3)

Ready to use. **Do not heat treat.** Store unused negative control at 2-8°C.

4.2.5 Conjugate (Reagent 4)

Ready to use. Store unused conjugate at 2-8°C.

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4.2.6 Washing buffer concentrate (Reagent ♦)

Prepare working strength washing buffer by adding 1 part of Reagent ♦ to 9 parts of fresh deionised or distilled water. Sufficient concentrate is provided to prepare up to 50mL working strength washing buffer for each strip of 8 wells. **Prepare fresh working strength washing buffer as required on the day of use** (See Section 6.2.10). Store remaining concentrate at 2-8°C.

4.2.7 Amplifier A (Reagent A)

Ready to use. Store unused amplifier A at 2-8°C.

4.2.8 Amplifier B (Reagent B)

Ready to use. Store unused amplifier B at 2-8°C.

4.2.9 Stopping solution (Reagent X)

Ready to use. Store unused stopping solution at 2-8°C.

5 ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED

5.1 REAGENTS

Fresh deionised or distilled water for preparation of working strength transport medium and washing buffer.

5.2 ACCESSORIES AND EQUIPMENT

IDEIA™ Chlamydia Specimen Collection Kits (S6007) and DAKO Chlamydia Dry Collection Kit (S6044) are recommended for collection of urethral and endocervical specimens to be tested using IDEIA™ PCE Chlamydia. Contact your local DAKO subsidiary or distributor for further information.

Clean, heat resistant (100°C), screw-capped vials.

Vortex mixer.

Waterbath or dry heating block to maintain heat at 95-100°C.

Precision pipettes and disposable tips to deliver 50µL-1,000µL or graduated pastettes for dispensing 200µL specimen (optional).

Bench-top centrifuge suitable for 2,500-3,500g (**required only for urine specimens**).

Centrifuge tubes or universal containers (**required only for urine specimens**).

Microtitration plate shaker capable of a minimum speed of 500 rpm with an orbital diameter of 1-3 mm. For information on suitability of plate shakers contact your local DAKO subsidiary or distributor.

Automated plate washer (optional) or suitable equipment for washing 8-well microtitration strips (Section 8.4.4).

Note: If washing less than 8 test wells in a strip using an automated washer with an 8-well head, it is important to completely fill the strip with blank wells.

Spectrophotometer or EIA plate reader able to read a 96-well microtitration plate of 8 well strips at an absorbance of 490nm with a reference at 620-650nm. (Optional, see Section 8.5, Reading the Test Results).

If verification of specimens shown to be reactive in the IDEIA™ PCE Chlamydia test is considered necessary, use of the IDEIA™ PCE Blocking Reagents (DAKO Product Code No S6041) is recommended. The blocking test for verification of results offers an additional means of quality controlling aspects of specimen collection.

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6 PRECAUTIONS

For *in vitro* diagnostic use. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.

6.1 SAFETY PRECAUTIONS

6.1.1 The following reagents contain sodium azide (15mmol/L) which is a poison; conjugate, washing buffer concentrate, positive control, negative control and amplifier A reagent. Sodium azide may react with copper and lead plumbing systems to form explosive metal azides. Always dispose of azide-containing materials by flushing with large quantities of water.

6.1.2 The stopping solution (Reagent X) contains 1mol/L phosphoric acid which is an irritant. Avoid eye and skin contact by wearing protective clothing and eye protection.

6.1.3 The positive control contains inactivated Chlamydia antigen which has been shown to be non-infectious. However, the control must be handled and disposed of as though potentially infectious.

6.1.4 The positive control contains formalin (0.1% v/v). If this reagent comes into contact with skin or mucous membranes wash immediately with large amounts of water.

6.1.5 Do not eat, drink, store or prepare foods, or apply cosmetics within the designated work area.

6.1.6 Do not pipette materials by mouth.

6.1.7 Wear disposable gloves while handling clinical specimens and always wash hands after working with infectious materials.

6.1.8 Dispose of all clinical specimens in accordance with local legislation.

6.2 TECHNICAL PRECAUTIONS

6.2.1 Components must not be used after the expiry date printed on the labels.

6.2.2 The reagents are provided at fixed working concentrations. Test sensitivity will be adversely affected if reagents are modified or stored under conditions other than those detailed in Section 4.2.

6.2.3 Do not mix or interchange different batches/lots of reagents.

6.2.4 The Positive Control is ready to use and must not be boiled. It has been previously heat treated and reheating may adversely affect the reagent.

6.2.5 Avoid contamination of reagents when opening and removing aliquots from the reagent bottles.

6.2.6 For small batches of tests it is recommended that the dropper bottle method is used for addition of amplification reagents to wells.

6.2.7 The use of swabs other than those supplied by DAKO should be checked for suitability by contacting your local DAKO subsidiary or distributor.

6.2.8 Use separate disposable pipettes or pipette tips for each sample, control or reagent (if not using dropper bottles) in order to avoid cross contamination of either samples, controls or reagents which could cause erroneous results. It is important to use clean pipette tips for dispensing amplifier B in order to reduce contamination of remaining reagent.

6.2.9 Store deionised or distilled water for dilution of concentrated reagents in clean containers to prevent microbial contamination.

6.2.10 Do not store unused working strength washing buffer for subsequent use. When not in use washing buffer reservoirs should be rinsed with deionised or distilled water and left to dry.

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6.2.11 Ensure that no cross-contamination occurs between wells at all stages of the test. It is essential that the polymer conjugate is not allowed to contaminate other reagents or equipment. If the dropper method is not used then reserve a separate pipette for dispensing conjugate and a separate pipette for dispensing the amplifier reagents. Avoid touching or splashing the rim of the well with conjugate. Conjugate allowed to dry on to the rim of the well may adversely affect the performance of the test.

6.2.12 The enzyme amplification system is a highly sensitive detector of alkaline phosphatase molecules. **It is very important that all non-bound conjugate is removed by thorough washing of wells before addition of amplification reagents.** Careful washing of wells is achieved by using techniques detailed in section 8.4.4. Inefficient washing may lead to incorrect results.

6.2.13 Manual or automated washing equipment must be free of microbial contamination, be correctly calibrated and maintained according to the manufacturer's instructions.

6.2.14 Phosphate Buffered Saline (PBS) and other wash solutions containing phosphate must not be used with the assay, to prevent inhibition of the conjugate enzyme which may affect test sensitivity. Washing equipment used with phosphate based wash solutions must be thoroughly flushed using distilled or deionised water prior to priming with working strength IDEIA™ PCE Chlamydia washing buffer.

6.2.15 Testing of specimens contaminated with faecal material in the IDEIA™ PCE Chlamydia kit has not been validated.

6.2.16 Do not re-use microwells.

7 COLLECTION AND PREPARATION OF SPECIMENS

Specimens collected in the following way can be treated in the IDEIA™ PCE Chlamydia Kit:

Dry swab specimens

Specimens collected in IDEIA™ PCE Chlamydia working strength transport medium.

7.1 TRANSPORT MEDIUM PREPARATION

Prior to specimen collection the transport medium concentrate (Reagent 1) should be diluted, dispensed and stored as described in Section 4.2.2.

7.2 SPECIMEN COLLECTION

Chlamydiae are intracellular organisms that infect the columnar epithelial surfaces¹ of the human urethra and endocervix. Specimens collected from these sites must contain as many columnar epithelial cells as possible.

The DAKO Chlamydia Dry Collection Kit (Code No. S6044) is recommended for the dry collection of endocervical and urethral specimens to be tested using IDEIA™ PCE Chlamydia.

Dry specimens may be transported for up to 72 hours at room temperature (15-30°C) prior to addition of 1mL working strength transport medium. After addition of transport medium specimens can be stored at 2-8°C for a further 5 days before testing.

The IDEIA™ Chlamydia Specimen Collection Kit (Code No. S6007) is recommended for the wet collection of urethral and endocervical specimens to be tested using IDEIA™ PCE Chlamydia.

Specimens may be transported for up to 48 hours at room temperature (15-30°C) prior to storage at 2-8°C for a further 5 days before testing.

Urethral specimen

Swab the urethra by inserting an appropriate swab 2 - 4cm into the urethra. Rotate the swab several times and withdraw it from the urethra. Place the swab in 1mL working strength transport medium in a heat resistant

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(100°C) vial or alternatively place the dry swab into a dry swab collection vial. Specimens may be stored at 2-8°C for no longer than 7 days prior to testing.

Endocervical specimen

Before sampling the endocervix clean the cervical os with sterile gauze to remove excess mucus/blood/pus etc. Swab the endocervix by inserting an appropriate swab approximately 1cm into the cervical canal and rotating the swab several times. Withdraw the swab without touching the vaginal surfaces and place in 1mL working strength transport medium in a heat resistant vial or alternatively place the dry swab into a dry swab collection vial. Specimens may be stored at 2-8°C for no longer than 7 days prior to testing.

Note: Water soluble lubricants should not be used during collection of endocervical swabs. In addition rectal, ano-rectal or specimens contaminated with faecal material must not be tested.

Urine specimen (males)

Collect approximately 20mL of first voided urine into a sterile container. Urine may be stored at 2-8°C for up to 3 days (or at -20°C for up to 4 weeks) before testing. Prior to testing, centrifuge urine at approximately 2500g to 3000g for 15 minutes using a bench top centrifuge. Remove and discard supernatant urine. Resuspend the deposit in 1mL of working strength transport medium in a heat resistant (100°C) vial. Urine deposit in transport medium may be stored for up to 7 days at 2-8°C prior to testing. Boric acid, at normal concentration used as a bacteriostatic agent in urine, has been shown not to affect the performance of the IDEIA™ PCE Chlamydia test.

Note: Patient should not have urinated for at least 1 hour prior to collection of urethral specimens and/or urine from males. For optimal detection of Chlamydial antigen in urine, a first catch urine is recommended.

7.3 PROCESSING OF SPECIMENS

7.3.1 Dry Swabs

Add 1mL of working strength transport medium to dry swab. Secure cap and vortex for 1 minute and proceed to section 8.2. Specimens may be stored at 2-8°C for no longer for seven days prior to testing.

7.3.2 Swabs in IDEIA™ PCE Chlamydia Transport Medium

Vortex samples prior to testing and proceed to section 8.2

8 TEST PROCEDURE

PLEASE REFER TO SECTION 6.2 TECHNICAL PRECAUTIONS BEFORE PERFORMING TEST PROCEDURE.

8.1 PREPARATION OF CONTROLS

Positive control (Reagent 2)

Vortex mix the working strength positive control for **1 minute**. Add the reagent directly to the appropriate well. **Do not heat treat positive control.** Positive control has been heat treated previously and reheating may adversely affect the reagent. If required, an additional control with a lower level of reactivity may be tested to monitor kit performance.

Negative control (Reagent 3)

Vortex mix the negative control for a **minimum of 15 seconds**. Add the reagent directly to appropriate wells. **Do not heat treat negative control.**

8.2 TREATMENT OF SPECIMENS

Specimens must be heat treated at 95 -100°C for 15 minutes prior to testing. This is best achieved using either a boiling waterbath or dry heat block as outlined below:

Boiling Waterbath

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Mix all specimens (ie transport medium containing swab or urine deposit) for a minimum of 15 seconds using a mechanical vortexer (see Section 7.2 for details of specimen collection). Place in a boiling water bath for 15 minutes. After 15 minutes remove vials and cool to room temperature (15-30°C). Vortex mix the specimens for at least 15 seconds immediately prior to testing.

Dry Heating Block

Dry baths or heating blocks should be allowed to pre-heat until a constant reading of 105°C is recorded by a metal probe thermometer placed directly in the heating chamber. This should ensure that the temperature inside the vials will be maintained at 95-100°C. Mix all specimens (ie transport medium containing swab or urinary deposit) for a minimum of 15 seconds using a mechanical vortexer (see Section 7.2 for details of specimen collection). When the temperature of the heating bath or block is stable at 105°C, heat the specimens for 20 minutes. After 20 minutes remove the specimens from the heat source and allow to cool to room temperature (15-30°C) before testing. Vortex mix the specimens for at least 15 seconds immediately prior to testing.

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Specimens must **NOT** be heated at higher temperatures or for longer periods than those described, as this may have an adverse effect on their performance in the assay.

8.3 STORAGE OF TREATED SPECIMENS

Specimens may be stored at -20°C for up to 4 weeks after heat treatment. If testing specimens that have been frozen, allow to thaw to room temperature (15-30°C) then vortex vigorously for a minimum of 1 minute immediately prior to testing. **Do not re-heat specimens.**

8.4 ASSAY PROCEDURE

NOTE: The assay procedure requires the use of a microtitration plate shaker. For information on suitability of shakers contact your local DAKO subsidiary or distributor.

It is recommended that consistent methods, for additions of reagents to the microtitration wells, are used throughout the test procedure ie: either pipette tips, dropper bottles or automated probes. For small batches of tests the use of the reagent dropper bottles avoids multiple entry of pipette tips to reagent bottles.

8.4.1 Sample and control addition

Locate the required number of wells into the well holder (plate). Add 200µL of heat treated specimens to the appropriate wells. Add 4 drops (or 200µL) of positive control (Reagent 2), and negative control (Reagent 3) to separate wells. (At least three negative control wells and one positive control well should be included with each batch of specimens tested).

8.4.2 Conjugate addition

After addition of all samples and controls add 2 drops (or 50µL) of conjugate (Reagent 4) to each well. **Avoid dipping pipette or dropper bottle tip into wells when dispensing conjugate as this may lead to cross-contamination between wells. Also avoid touching or contaminating tops or rims of microtitration wells with conjugate as this may adversely affect the performance of the test.**

8.4.3 Sample and conjugate incubation

Incubate the wells on a shaker at room temperature (15-30°C) with **shaking for 90 minutes.**

8.4.4 Washing the wells

The wells should be washed using freshly prepared working strength washing buffer. (See Section 4.2.6). The washing technique is critical to the test performance (**Section 6.2.12**) and should be carried out so as to ensure complete filling (with a minimum of **350µL** of working strength washing buffer) and emptying of the wells. At least four wash cycles are essential, by either automated or manual washing techniques, which should include a 2 minute soak period during the second wash or a total of a 2 minute soak period during the complete cycle.

Manual washing

If washing wells manually, aspirate or shake out the contents of the wells and using freshly prepared washing buffer, ensure complete filling and emptying of wells. Between each wash step remove all remaining wash buffer by tapping the inverted wells on to clean absorbent material. Manual washing efficiency is further ensured if the washing buffer is delivered at an angle so as to produce a vortex in the wells.

Automated washing

Automated washers should be programmed to complete at least 4 wash cycles and to incorporate the equivalent of 2 minutes soaking time during the complete washing cycle. Washers must be correctly calibrated to ensure complete filling and emptying of wells between each wash.

8.4.5 Amplifier addition and incubation

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Add 2 drops (or 100 μ L) of amplifier A (Reagent A) to each well.

Add 2 drops (or 100 μ L) of amplifier B (Reagent B) to each well.

Avoid touching wells with dropper tips or pipette tips when dispensing amplifier A and B as this may lead to cross-contamination between wells.

8.4.6 Second Incubation

Incubate the wells on a shaker at room temperature (15-30°C) with **shaking** for **30 minutes**.

8.4.7 Stopping the reaction

Add 2 drops (or 100 μ L) of stopping solution (Reagent X) to each well. Ensure thorough mixing in the wells. The coloured product is stable for 30 minutes. **Do not expose to direct sunlight** as photobleaching of the coloured product may occur.

8.5 READING THE TEST RESULTS

8.5.1 Photometric reading

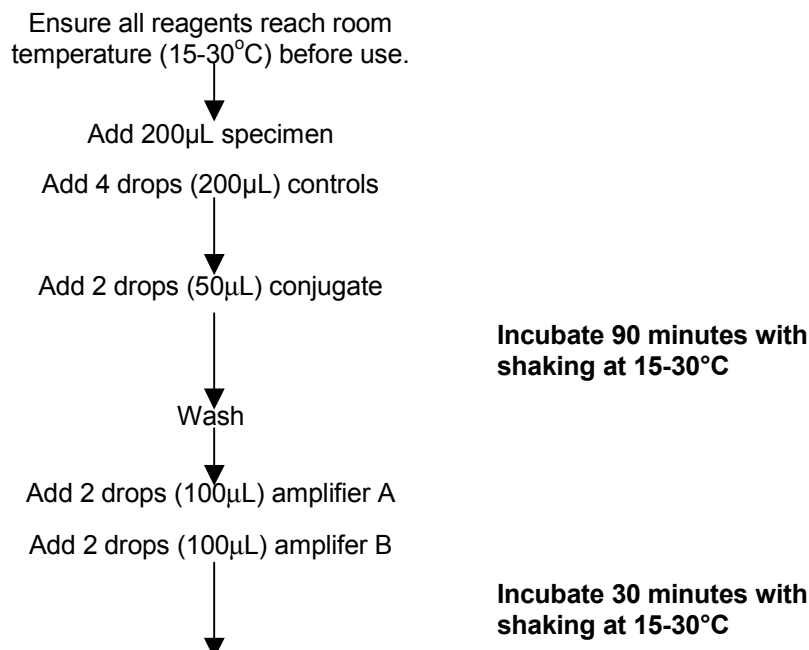
The wells should be read photometrically within 30 minutes after addition of the stopping solution. Mix the contents of the microwells and read the absorbance of each well using a suitable spectrophotometer or EIA plate reader set at 490nm. Ensure that the bottoms of the wells are clean before reading and check that no foreign matter is present in the wells. The reader should be blanked on air (i.e. with no plate in the carriage) before the plate is scanned.

Alternatively if the spectrophotometer or EIA plate reader allows for the use of a reference wavelength (at 620 to 650nm), dual wavelength reading should be performed which will eliminate any potential interference caused by aberrations, such as dirt or marks, on the optical surface of the wells.

8.5.2 Visual reading

The wells may be assessed visually up to 30 minutes after addition of the stopping solution. It is recommended that wells in which the colour intensity is difficult to interpret when compared to the negative control are also read photometrically. (See Section 9.3).

Diagram 1: Summary of IDEIA™ PCE Chlamydia Assay Procedure



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Add 2 drops (100 μ L) stopping solution



Read absorbance at 490nm

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9 **QUALITY CONTROL AND INTERPRETATION OF TEST RESULTS**

9.1 **NEGATIVE CONTROL**

As detailed in Section 8.4.1 (Sample and control addition), three negative control wells must be included in each assay.

Photometric determination

Individual negative control absorbance values must be less than or equal to 0.20 absorbance units. Individual negative control absorbance values must fall within ± 0.05 absorbance units of the mean of the three negative controls. If one negative control absorbance value falls outside the accepted range, exclude this value and recalculate the mean of the remaining two. If two negative control absorbance values are unacceptable the test must be repeated.

Calculation of the Cut-Off Value

The cut-off value is calculated by adding 0.05 to the mean negative control absorbance value.

Visual determination

All negative control wells should be colourless or only slightly pink. If this is not the case test results should not be determined visually. Results should be read photometrically or the test repeated.

9.2 **POSITIVE CONTROL**

As detailed in section 8.4.1 (Sample and control addition) one positive control well must be included in each assay.

Photometric determination

The positive control well must have an absorbance value of greater than 0.50 absorbance units. If this is not the case the test should be repeated.

Visual determination

The positive control well should be a red/magenta colour clearly distinguishable from the negative controls. If this is not the case the test results should not be determined visually. Results should be read photometrically or the test repeated.

9.3 **SPECIMENS**

Photometric determination

Clinical specimens having absorbance values greater than the cut-off value are positive (see Section 9.1). A result within 0.015 absorbance units of the cut-off value should be interpreted cautiously, and the test repeated or the patient resampled. Patient results should not be reported if controls are outside the expected values.

Visual determination

Any specimen giving a red/magenta colour more intense than that of the negative controls is positive. Any specimen giving colour equal to or less than the colour of the negative controls is negative. Any specimen giving a pale pink colouration close to that of the negative controls should be read photometrically or retested. Alternatively the patient should be resampled.

9.4 **VERIFICATION OF RESULTS**

Positive and equivocal results may be re-tested with the IDEIA™ PCE Chlamydia Blocking Reagents (Code S6041) to verify the presence of chlamydial antigen.

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Table 1
Summary of Interpretation of Results and Recommended Reporting

Result	Interpretation	Reporting Recommendations
OD > CO + 0.015	Positive*	Presumptive chlamydial LPS antigen (No blocking test performed)
OD = CO ± 0.015	Equivocal*	Unable to determine result. Retest
OD < CO - 0.015	Negative	No chlamydial LPS antigen detected

OD = Optical Density (Absorbance units)

CO = Cut off = Mean of Negative Controls + 0.05 Absorbance units

* Positive and equivocal results may be re-tested using the IDEIA™ PCE Chlamydia Blocking Reagents (Code S6041) to verify the presence of chlamydial LPS.

10 PERFORMANCE LIMITATIONS

10.1 Specimen quality is crucial to the success of all diagnostic tests. Specimens collected from human urethral and endocervical sites must contain as many columnar epithelial cells as possible. Some specimens will contain Chlamydia organisms at levels below the detection limit of the IDEIA™ PCE Chlamydia test and will therefore give negative results.

10.2 The IDEIA™ PCE Chlamydia test should be used to test only human urethral, endocervical or urine (male) specimens. The testing of specimens from other sites has not been validated.

10.3 In situations where a male urethral swab cannot be taken then a urine specimen may be collected. For urine and urethral specimen collection the patient should not have urinated for at least 1 hour prior to specimen collection.

10.4 The swabs provided in the IDEIA™ Chlamydia Specimen Collection kits and DAKO Chlamydia Dry Collection kits have Dacron tips and metal and/or plastic shafts. Other types of swab have not been validated. **Wooden stemmed, calcium alginate or agar-or charcoal-containing swabs must not be used.**

10.5 Use of water soluble lubricants during sample collection – water soluble lubricants should not be used when collecting endocervical swabs from patients with a suspected *Chlamydia trachomatis* infection. The use of water soluble lubricants, for personal or gynaecological use, can give rise to false reactions in tests used for diagnosing *Chlamydia trachomatis* infections. Samples collected in this way will be confirmed as false positive when tested in DAKO's Chlamydia ELISA test in conjunction with IDEIA™ PCE Chlamydia Blocking test.

10.6 Antigen detection systems, such as IDEIA™ PCE Chlamydia, should not be used to provide information for medico-legal cases. Only standard Chlamydia cultures should be used in the evaluation of suspected abuse or other situations in which the possibility of a false positive result with antigen detection systems is unacceptable¹⁰.

10.7 In patient populations with low disease prevalence, the results of antigen detection systems should be interpreted cautiously.

10.8 No data is available on the use of IDEIA™ PCE Chlamydia in the determination of patients' response to therapy.

10.9 The monoclonal antibody used in the IDEIA™ PCE Chlamydia test is genus specific and will not differentiate between Chlamydia species.

10.10 The conjugate monoclonal antibody used in the IDEIA™ PCE Chlamydia test has been shown not to bind to Protein A producing strains of *Staphylococcus aureus* at a concentration of 10⁷ CFU/mL.

10.11 Occasionally patients who have received preparations of mouse monoclonal antibodies for diagnostic and therapeutic purposes may develop human anti-mouse antibodies. Specimens from patients known to have developed such antibodies should not be tested in antigen detection tests based on the use of murine monoclonal antibodies.

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10.12 Test results should be interpreted in conjunction with information available from epidemiological studies, clinical assessment of the patient and other diagnostic procedures.

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11 EXPECTED VALUES

The prevalence of Chlamydia infections in a study population will vary according to the type of population studied¹. The prevalence of urino-genital Chlamydia infection for unselected patients attending a genito-urinary medicine clinic will vary between 10% to 25%. For patients attending with non-specific urethritis and post-gonococcal urethritis the prevalence may be as high as 30% to 60%.

A lower prevalence of urino-genital infection (less than 10%) will be found in populations of patients attending obstetric and gynaecology clinics^{1,11}.

12 SPECIFIC PERFORMANCE CHARACTERISTICS

12.1 CLINICAL STUDIES

The IDEIA™ *PCE* Chlamydia test was evaluated in clinical studies performed at three centres in the UK.

Studies were conducted on urethral, endocervical and urine specimens taken from 987 patients (605 males, 382 females) attending genitourinary medicine clinics (high risk group; incidence of infection by reference methods being 10.0%).

Studies were also performed on endocervical specimens taken from 539 female patients attending antenatal clinics (low risk group, incidence of infection by reference methods being 5.4%).

The reference methods used to determine *Chlamydia trachomatis* infection were IDEIA™ Chlamydia with verification by either direct immunofluorescence and/or a chlamydia specific blocking test.

12.1.1 Performance

Endocervical and Urethral Specimens

The results of these studies are shown in Table 12.1.

Overall, IDEIA™ *PCE* Chlamydia agreed with the reference methods for 1043 out of 1047 specimens, giving **agreement of 99.6%**.

The relative **sensitivity** of IDEIA™ *PCE* Chlamydia for the **high risk** and **low risk** groups was **100%** (60/60 high risk : 29/29 low risk).

The relative **specificity** of IDEIA™ *PCE* Chlamydia for the **high risk** and **low risk** groups was **100%** (448/448) and **99.2%** (506/510) **respectively**.

Overall the relative sensitivity and relative specificity of IDEIA™ *PCE* Chlamydia was 100% (89/89) and 99.6% (954/958) respectively.

The positive predictive value of IDEIA™ *PCE* Chlamydia test, for the sample size and populations tested, was 100% (60/60) and 100% (33/33) for high and low risk groups respectively.

The negative predictive value of IDEIA™ *PCE* Chlamydia test, for the sample size and populations tested, was 100% (448/448) and 99.2% (506/510) for high and low risk groups respectively.

Overall the predictive values for positive and negative IDEIA™ *PCE* Chlamydia test results were 100% (89/89) and 99.6% (954/958) respectively.

Urine Specimens (Males)

The results of these studies are shown in Table 12.1. Overall IDEIA™ *PCE* Chlamydia agreed with the reference methods for 474 specimens out of 479 specimens, giving **agreement of 99.0%**.

The relative **sensitivity** and relative **specificity** of IDEIA™ *PCE* Chlamydia was **97.5%** (39/40) and **99.1%** (435/439) **respectively**.

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The predictive values for positive and negative IDEIA™ *PCE* Chlamydia test results on male urine samples were **97.7%** (43/44) and **99.1%** (435/439) respectively.

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Overall Performance

Overall the **relative sensitivity** and **relative specificity** of IDEIA™ *PCE* Chlamydia was **99.2%** (128/129) and **99.4%** (1389/1397) respectively.

The **prevalence rate** of Chlamydia detection by the IDEIA™ *PCE* Chlamydia test **increased from 9.0% to 10.4%** for the **high risk groups** and from **5.2% to 6.1%** for the **low risk group** when compared to IDEIA™ Chlamydia test results. The **overall prevalence rate** of chlamydia antigen detection by the IDEIA™ *PCE* Chlamydia test was found to be **8.9% compared to 7.7%** for the IDEIA™ Chlamydia test.

Using this data a predictive reassignment of the sensitivity and specificity of IDEIA™ *PCE* Chlamydia compared to cell culture was performed (Table 12.2) based on the comparative data for IDEIA™ Chlamydia.

Table 12.1 Comparison of Test Results by IDEIA™ *PCE* Chlamydia and Reference Methods (IDEIA™ Chlamydia, Immunofluorescence and Blocking test) on Urethral, Endocervical and Urine Specimens in High and Low Risk Populations.

Sample	Population	Prevalence Rate %		Sensitivity %	Specificity %
		IDEIA™ Chlamydia	IDEIA™ <i>PCE</i> Chlamydia		
Urethral	High Risk	13.5 (17/126)	15.1 (19/126)	100 (19/19)	100 (107/107)
Endocervical	High Risk	8.6 (33/382)	10.7 (41/382)	100 (41/41)	100 (341/341)
	Low Risk	5.2 (28/539)	6.1 (33/539)	100 (29/29)	99.2 (506/510)
Urines (male)	High Risk	8.1 (39/479)	9.0 (43/479)	97.5 (39/40)	99.1 (435/439)
Overall Total		7.7 (117/1526)	8.9 (136/1526)	99.2 (128/129)	99.4 (1389/1397)

Table 12.2 Correlation of IDEIA™ Chlamydia and IDEIA™ *PCE* Chlamydia with Cell Culture

Sample	Population	IDEIA™ Chlamydia ¹		IDEIA™ <i>PCE</i> Chlamydia ²	
		Sensitivity (%)	Specificity (%)	Predicted Sensitivity (%)	Predicted Specificity (%)
Urinogenital	High Risk	91.9% (385/419)	98.5% (1806/1834)	100% ³	98.5% ⁴
	Low Risk	100% (40/40)	98.6% (575/583)	100% ³	97.8% ⁴
Urines (Male)	High Risk	84.7% (149/176)	98.7% (938/950)	93.38% ³	97.8% ⁴

1. Based on external evaluations performed at four centres.
2. Predictive assignment based on comparative data with IDEIA™ Chlamydia (Table 12.1).
3. Predicted value calculated from increased positivity rate obtained with IDEIA™ *PCE* Chlamydia compared to IDEIA™ Chlamydia (Table 12.1).
4. Predicted value calculated from specificity for IDEIA™ *PCE* Chlamydia data versus reference method (Table 12.1).

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12.3 CROSS REACTIVITY

The following overnight broth cultures were found to be non-reactive with the IDEIA™ PCE Chlamydia test.

<i>Acholeplasma laidlawii</i>	<i>Mycoplasma arginini</i>
<i>Acinetobacter calcoaceticus var anitratus</i>	<i>Mycoplasma hyorhinis</i>
<i>Aeromonas hydrophila</i>	<i>Mycoplasma pneumoniae</i>
<i>Bacteroides fragilis</i>	<i>Mycoplasma salivarium</i>
<i>Bacillus cereus</i>	<i>Mycoplasma genitalium</i>
<i>Campylobacter coli</i>	<i>Mycoplasma fermentans</i>
<i>Candida albicans</i>	<i>Neisseria gonorrhoeae</i>
<i>Citrobacter freundii</i>	<i>Peptococcus sp</i>
<i>Clostridium perfringens</i>	<i>Peptostreptococcus anaerobius</i>
<i>Clostridium difficile</i>	<i>Proteus mirabilis</i>
<i>Enterobacter cloacae</i>	<i>Pseudomonas aeruginosa</i>
<i>Enterococcus faecalis</i>	<i>Salmonella minnesota</i>
<i>Escherichia coli</i>	<i>Serratia marcescens</i>
<i>Gardnerella vaginalis</i>	<i>Shigella sonnei</i>
<i>Haemophilus influenzae</i>	<i>Staphylococcus epidermidis</i>
<i>Klebsiella aerogenes</i>	<i>Staphylococcus aureus</i>
<i>Lactobacillus lactis</i>	<i>Streptococcus pyogenes</i>
<i>Listeria monocytogenes</i>	<i>Ureaplasma sp</i>
<i>Mycoplasma orale</i>	<i>Veillonella spp</i>
<i>Mycoplasma hominis</i>	