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IDEIA *PCE* Chlamydia

REF K603211-2 ..... Σ 192 **EN**

A dual amplification immunoassay for the detection of Chlamydia antigen in clinical specimens.

1. INTENDED USE

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

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 10^4 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 subacute 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 immunofluoresence 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 immunoassays[4]. Other methods include techniques such as the polymerase chain reaction for detection of Chlamydia nucleic acids[5].

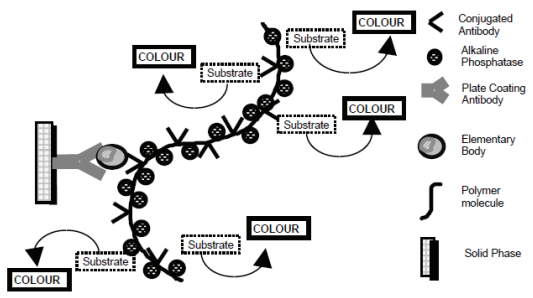
Culture techniques are expensive and require specialised laboratory facilities. Methods for detection of nucleic acids also require specialised equipment and laboratory facilities to minimise specificity problems. Direct detection by immunofluorescence staining becomes labour intensive when used to routinely 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 Oxoid's patented enzyme amplification technology[4]. The presence of Chlamydia antigen in clinical specimens is indicated by a coloured end-point.

* US Patent No. 5,543,332

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 ratio[9] and a liquid, ready to use enzyme amplification system[4]. 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. DEFINITIONS

The following symbols have been used throughout the product information:

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
Σ N	Contains sufficient for <N> tests
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

5. REAGENTS PROVIDED

Σ 192 - Each kit contains sufficient materials for 192 determinations.

The shelf life of the kit is as indicated on the outer box label

5.1. IDEIA *PCE* CHLAMYDIA TEST CONTENTS

One Instructions For Use booklet

MICROTITRATION PLATE — Two 96 well Microtitration Plates of 12, 8 microwell break-apart strips coated with Chlamydia specific (anti-lipopolysaccharide) monoclonal antibody. A resealable plastic pouch is provided for storage of unused microwells.

One bottle of each of the following unless indicated otherwise:

TRANSPORT MEDIUM (X10) — 25mL Transport Medium concentrate (x10): nonionic detergent in a buffer containing coloured dye, antimicrobial agent and an antifoaming agent

CONTROL + — 7mL Positive Control: heat inactivated Chlamydia antigen in buffer solution containing formalin, antimicrobial agent and coloured dye

CONTROL – — 12mL Negative Control: buffer solution containing antimicrobial agent, coloured dye and an anti-foaming agent

CONJUGATE — 2x 7mL polymer Conjugate: Chlamydia specific (antilipopolysaccharide) monoclonal antibody Conjugated to a dextran polymer backbone linked to multiple alkaline phosphatase molecules in stabilising buffer containing coloured dye and an antimicrobial agent

WASH BUFFER (X20) — 2x 125mL Wash Buffer concentrate (x20): tris buffered solution containing detergent and an antimicrobial agent

AMPLIFIER A — 2x 13mL Amplifier A: inorganic salts and buffered enzyme solution containing tetrazolium violet and an antimicrobial agent

AMPLIFIER B — 2x 13mL Amplifier B: stabilised NADPH solution

STOP SOLUTION — 2x 13mL Stop Solution: 1 mol/L phosphoric acid

5.2. PREPARATION, STORAGE AND RE-USE OF KIT COMPONENTS

The IDEIA *PCE* Chlamydia kit format allows for testing of up to 24 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:

5.2.1 Monoclonal Antibody Coated Microwells - MICROTITRATION PLATE — 2°C–8°C

Open the plate pouch by cutting along the seal. Break off the required number of microwells and relocate them into the frame. Place unused microwells back into the plate pouch along with the dessicant, place pouch inside plastic bag and carefully seal, store at 2-8°C. Microwells may be used for up to 6 weeks after initial opening, provided they are stored in this manner.

5.2.2 Transport Medium Concentrate - TRANSPORT MEDIUM (X10) — 2°C–8°C

It is important to thoroughly mix the concentrated Transport Medium prior to diluting it to working strength.

An anti-foaming agent in Transport Medium concentrate 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 Transport Medium concentrate 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).

5.2.3 Positive Control - CONTROL + — 2°C–8°C
Ready to use. **Do not heat treat**. Store unused Positive Control at 2-8°C

5.2.4 Negative Control - CONTROL – — 2°C–8°C
Ready to use. **Do not heat treat**. Store unused Negative Control at 2-8°C

5.2.5 Conjugate CONJUGATE — 2°C–8°C
Ready to use. Store unused Conjugate at 2-8°C.

5.2.6 Wash Buffer Concentrate - WASH BUFFER (X20) — 2°C–8°C
Provided x20 concentrate. Prepare working strength Wash Buffer by adding 1 part of Wash Buffer concentrate to 19 parts of fresh deionised or distilled water. Sufficient concentrate is provided to prepare up to 100mL working strength Wash Buffer for each strip of 8 microwells. **Prepare working strength Wash Buffer as required on the day of use** (See Section 8.2.12).
Store remaining concentrate at 2-8°C.

Do not store unused working strength Wash Buffer for subsequent use (See Section 8.2.12).

5.2.7 Amplifier A - AMPLIFIER A — 2°C–8°C
Ready to use.
Store unused Amplifier A at 2-8°C.

5.2.8 Amplifier B - AMPLIFIER B — 2°C–8°C
Ready to use.
Store unused Amplifier B at 2-8°C.

5.2.9 Stop Solution - STOP SOLUTION — 2°C–8°C
Ready to use.
Store unused Stop Solution at 2-8°C.

6. ADDITIONAL REAGENTS

6.1. REAGENTS

Fresh deionised or distilled water for preparation of working strength Transport Medium and wash buffer.

6.2. ACCESSORIES

The following products are intended for use in conjunction with IDEIA *PCE* Chlamydia. Contact your local distributor for further information

IDEIA Chlamydia Specimen Collection Kit (REF S600730-2) is intended for collection of urethral and endocervical specimens to be tested using IDEIA *PCE* Chlamydia.

The kit performance may be adversley affected by the use of collection kits or swabs other than those specified

IDEIA *PCE* Chlamydia Transport Medium 25mL (REF S603830-2).

IDEIA *PCE* Chlamydia/HSV Wash Buffer Concentrate (X20) 125mL (REF S603930-2).

IDEIA *PCE* Chlamydia Blocking Reagents (REF S604130-2)

7. EQUIPMENT

The following equipment is required:

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

Vortex mixer

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

Clean absorbent paper (onto which microwells can be tapped dry)

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

Waste discard container with suitable fresh disinfectant

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 1-3mm. For information on suitability of plate shakers contact your local distributor.

Automated plate washer (optional) or suitable equipment for washing 8 microwell strips (See Section 10.4.4). **Note: If washing less than 8 test microwells in a strip using an automated washer with an 8 microwell head, it is important to completely fill the strip with blank microwells**.

Spectrophotometer or EIA plate reader able to read a 96 microwell plate of 8 microwell strips at an absorbance of 490nm with a reference at 620-650nm. (Optional, See Section 10.5, Reading the Test Results).

Application notes for use on open automated systems are available for this assay. Contact your local distributor.

8. PRECAUTIONS

IVD 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

8.1. SAFETY PRECAUTIONS

8.1.1 The following reagents contain sodium azide (<0.1%) which is a poison; Conjugate, Wash 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.

8.1.2 The Stop Solution contains 9.8% phosphoric acid. Avoid eye and skin contact by wearing protective clothing and eye protection.

8.1.3 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.

8.1.4 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.

8.1.5 The Transport Medium Concentrate contains 0.75% Proclin 300 which is classified per applicable European Economic Community (EEC) Directives as a hazard. The following are the appropriate hazard (H) and precautionary (P) statements.

WARNING

H317	May cause an allergic skin reaction
P280	Wear protective gloves/ protective clothing/ eye protection/face protection
P302+P352	IF ON SKIN: wash with plenty of soap and water
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention

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

8.1.7 Do not pipette materials by mouth.

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

8.1.9 Dispose of all clinical specimens and reagents in accordance with local legislation.

8.2. TECHNICAL PRECAUTIONS

8.2.1 Components must not be used after the expiry date printed on the labels. Do not mix or interchange different batches/lots of reagents.

8.2.2 The reagents are provided at fixed working concentrations. Test performance will be adversely affected if reagents are modified or stored under conditions other than those detailed in Section 5.2.

8.2.3 **The Positive Control and Negative Control are ready to use and must not be boiled.**

8.2.4 Avoid contamination of reagents.

8.2.5 The assay must be performed using pipettes and not dropper bottles.

8.2.6 Avoid multiple sampling from amplification reagents. Transfer the required amount into a suitable clean vessel. Do not return excess reagent to the bottle.

8.2.7 Use separate disposable pipettes or pipette tips for each specimen, control or reagent in order to avoid cross contamination of either specimens, controls or reagents which could cause erroneous results.

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

8.2.9 Ensure that no cross-contamination occurs between microwells at all stages of the test. It is essential that the polymer Conjugate is not allowed to contaminate other reagents or equipment. Reserve a separate pipette for dispensing Conjugate and a separate pipette for dispensing the Amplifier reagents. Avoid touching or splashing the rim of the microwell with Conjugate. Conjugate allowed to dry on to the rim of the microwell may adversely affect the performance of the test.

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

8.2.11 Microwells can not be re-used.

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

8.2.13 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 microwells before addition of amplification reagents.** Careful washing of microwells is achieved by using techniques detailed in Section 10.4.4. Inefficient washing may lead to incorrect results.

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

8.2.15 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 performance. 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/HSV Wash Buffer (X20) (REF S603930-2).

9. COLLECTION AND PREPARATION OF SPECIMENS

Specimens collected in the following way can be tested in the IDEIA *PCE* Chlamydia kit:

Dry swab specimens (See Section 10.2.1)

Specimens collected in IDEIA *PCE* Chlamydia working strength Transport Medium (See Section 10.2.2).

9.1. TRANSPORT MEDIUM PREPARATION.

Prior to specimen collection the Transport Medium concentrate should be diluted, dispensed and stored as described in Section 5.2.2.

Note: It is important to vortex or mix Transport Medium thoroughly prior to dispensing or use. The Transport Medium contains an antifoaming agent which causes the medium to appear cloudy. This does not affect the test and is not due to microbial contamination of the Transport Medium

9.2. SPECIMEN COLLECTION

Chlamydiae are intracellular organisms that infect the columnar epithelial surfaces[1] of the human urethra and endocervix. Specimens collected from these sites must contain as many columnar epithelial cells as possible.

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 (REF S600730-2) is intended 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 (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

10. TEST PROCEDURE

PLEASE REFER TO SECTION 8.2 TECHNICAL PRECAUTIONS BEFORE PERFORMING TEST PROCEDURE.

10.1. PREPARATION OF CONTROLS

Negative Control

Vortex mix the Negative Control for a minimum of **15 seconds**. Add the reagent directly to appropriate microwells. **Do not heat treat Negative Control**.

Positive Control

Vortex mix the Positive Control for **1 minute**. Add the reagent directly to the appropriate microwell. **Do not heat treat Positive Control**. If required, an additional control with a lower level of reactivity may be tested to monitor kit performance

10.2. TREATMENT OF SPECIMENS

10.2.1 Dry Swabs

Add 1mL of working strength Transport Medium to dry swab. Secure cap and vortex for 1 minute. If specimens are not to be tested immediately they can be stored at 2-8°C for up to 5 days

10.2.2 Swabs In IDEIA *PCE* Chlamydia Transport Medium

Specimens received in Transport Medium may be stored at 2-8°C for up to 7 days from date of collection

10.2.3 Heating 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

Mix all specimens (ie Transport Medium containing swab or urine deposit) for a minimum of 15 seconds using a mechanical vortexer (see Section 9.2 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 9.2 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.

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.

10.3. 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**

10.4. ASSAY PROCEDURE

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

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

10.4.1 Specimen and Control Addition

Locate the required number of microwells into the microwell holder. Add 200µL of heat treated specimens to the appropriate microwells. Add 200µL of Negative Control, and Positive Control to separate microwells. (At least three Negative Control microwells and one Positive Control microwell should be included with each batch of specimens tested).

10.4.2 Conjugate Addition

After addition of all specimen and controls add 50µL of Conjugate to each microwell. **Avoid dipping pipette tip into microwells when dispensing Conjugate as this may lead to cross-contamination between microwells. Also avoid touching or contaminating tops or rims of microwells with Conjugate as this may adversely affect the performance of the test**

10.4.3 First Incubation

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

10.4.4 Washing the Microwells

The microwells should be washed using freshly prepared working strength Wash Buffer (see Section 5.2.6).

The washing technique is critical to the test performance (see Section 8.2.12) and should be carried out so as to ensure complete filling (with a minimum of **350µL** of working strength wash buffer) and emptying of the microwells.

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 microwells manually, aspirate or shake out the contents of the microwells and using freshly prepared wash buffer, ensure complete filling and emptying of microwells. Between each wash step remove all remaining Wash Buffer by tapping the inverted microwells on to clean absorbent material. Manual washing efficiency is further ensured if the Wash Buffer is delivered at an angle so as to produce a vortex in the microwells. After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of wash buffer.

Automated Washing

Automated washers should be programmed to complete 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 microwells between each wash. After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of wash buffer

10.4.5 Amplifier Addition

Add 100µL of Amplifier A to each microwell.
Add 100µL of Amplifier B to each microwell.

Avoid touching microwells with pipette tips when dispensing Amplifier A and B as this may lead to cross-contamination between microwells

10.4.6 Second Incubation

Incubate the microwells on the plate shaker at room temperature (**15-30°C**) with **shaking** for **30 minutes**

10.4.7 Stopping the Reaction

Add 100µL of Stop Solution to each microwell. Ensure thorough mixing in the microwells. The coloured product is stable for 30 minutes. **Do not expose to direct sunlight** as photobleaching of the coloured product may occur

10.5. READING THE TEST RESULTS

10.5.1 Visual Reading

The microwells may be assessed visually up to 30 minutes after addition of the Stop Solution. It is recommended that microwells in which the colour intensity is difficult to interpret when compared to the Negative Control are also read photometrically (see Section 10.5.2)

10.5.2 Photometric Reading

The microwells should be read photometrically within 30 minutes after addition of the Stop Solution. Mix the contents of the microwells and read the absorbance of each microwell using a suitable spectrophotometer or EIA plate reader set at 490nm. Ensure that the bottoms of the microwells are clean before reading and check that no foreign matter is present in the microwells. The reader should be blanked on air (ie 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 microwells

10.6. SUMMARY OF IDEIA *PCE* CHLAMYDIA ASSAY PROCEDURE

Ensure all reagents reach room temerature (15-30°C) before use

↓

Add 200µL specimen
Add 200µL Positive and Negative Controls
CONTROL + | CONTROL −

↓

Add 50µL Conjugate CONJUGATE

↓ **Incubate 90 minutes with shaking at 15-30°C**

Wash (x4)

↓

Add 100µL Amplifier A AMPLIFIER A
Add 100µL Amplifier B AMPLIFIER B

↓ **Incubate 30 minutes with shaking at 15-30°C**

Add 100µL Stop Solution STOP SOLUTION

↓

Read absorbance at 490nm

11. QUALITY CONTROL AND INTERPRETATION OF TEST RESULTS

11.1. NEGATIVE CONTROL

As detailed in Section 10.4.1 (Specimen and Control Addition), three Negative Control microwells must be included in each assay.

Visual Determination

All Negative Control microwells 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.

Calculation of the Cut-Off Value

The cut-off value is calculated by adding 0.05 to the mean Negative Control absorbance value.

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

11.2. POSITIVE CONTROL

As detailed in Section 10.4.1 (Sample and Control Addition), one Positive Control microwell must be included in each assay.

Visual Determination

The Positive Control microwell 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.

Photometric Determination

The Positive Control microwell must have an absorbance value of greater than 0.50 absorbance units. If this is not the case the test should be repeated

11.3. SPECIMENS

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.

Photometric Determination

Clinical specimens having absorbance values greater than the cut-off value are positive (see Section 11.1). 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

11.4. INTERPRETATION AND VERIFICATION OF TEST RESULTS

11.4.1 Interpretation of Test Results

The following table is a summary of the recommended interpretation and reporting of results

Table 11.4 Summary of Intepretation of Results and Recommended Reporting

Result	Interpretation	Reporting Recommentations
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 should be verified

11.4.2 Verification of Test Results

It is strongly recommended that a verification method is used to confirm specimens shown to be reactive in IDEIA *PCE* Chlamydia test. The IDEIA *PCE* Chlamydia Blocking Reagents (REF S604130-2) are intended for verification of results, and offer an additional means of controlling quality aspects relating to specimen collection.

12. PERFORMANCE LIMITATIONS

12.1. 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 (see Section 9). Some specimens will contain Chlamydia organisms at levels below the detection limit of the IDEIA *PCE* Chlamydia test and will therefore give negative results.

12.2. 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.

12.3. 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.

12.4. The swabs provided in the IDEIA Chlamydia Specimen Collection kits and 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.**

12.5. **Use of water soluble lubricants during specimen 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. Specimens collected in this way will be confirmed as false positive when tested in IDEIA *PCE* Chlamydia in conjunction with IDEIA *PCE* Chlamydia Blocking test

12.6. 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 unacceptable[10].

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

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

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

12.10. Specimens containing Protein A producing strains of *Staphylococcus aureus* at a concentration of 10^7 CFU/mL. have been shown to be non-reactive in the IDEIA *PCE* Chlamydia test.

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

13. EXPECTED VALUES

Positivity rates may vary according to the prevalence of Chlamydia in different populations, geographical location, specimen collection, handling, storage transportation of specimens and the general health environment of the patient population under study[1]. The prevalence of urinogenital 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 postgonococcal 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].

14. SPECIFIC PERFORMANCE CHARACTERISTICS

14.1. 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.

14.1.1 Performance

Endocervical and Urethral Specimens

The results of these studies are shown in Appendix I.

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 **h**igh 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**.

Urine Specimens (Males)

The results of these studies are shown in Appendix I. 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.

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 (Appendix II) based on the comparative data for IDEIA Chlamydia

14.2. CROSS REACTIVITY

The following overnight broth cultures were found to be non-reactive with the monoclonal antibody used in the IDEIA *PCE* Chlamydia test

Bacteria

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

Viruses

Herpes simplex virus

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Appendix I: 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

Specimen	Population	Prevelance Rate %		No. specimens positive in IDEIA Chlamydia and IDEIA PCE Chlamydia	Sensitivity %	Specificity %
		IDEIA Chlamydia	IDEIA PCE Chlamydia			
Urethral	High Risk	**13.5** (17/126)	**15.1** (19/126)	17/126[1]	**100** (19/19)	**100** (107/107)
Endocervical	High Risk	**8.6** (33/382)	**10.7** (41/382)	33/382[2]	**100** (41/41)	**100** (341/341)
	Low Risk	**5.2** (28/539)	**6.1** (33/359)	28/539[3]	**100** (29/29)	**99.2** (506/510)
Urine (male)	High Risk	**8.1** (39/479)	**9.0** (43/479)	38/479[4]	**97.5** (39/40)	**99.1** (435/439)
Overall Total		**7.7** (117/1526)	**8.9** (136/1526)	116/1526	**99.2** (128/129)	**99.4** (1389/1397)

1. 3 specimens gave discrepant results, one specimen was IDEIA PCE Chlamydia negative and IDEIA Chlamydia positive, the specimen was negative in both assays on retesting. Two specimens were positive in IDEIA PCE Chlamydia and negative in IDEIA Chlamydia, both specimens were positive by direct immunofluorescence.
2. 8 specimens gave discrepant results, all positive in IDEIA PCE Chlamydia and negative in IDEIA Chlamydia. All 8 specimens were positive by direct immunofluorescence.
3. 5 specimens gave discrepant results, all positive in IDEIA PCE Chlamydia and negative in IDEIA Chlamydia. On testing by direct immunofluorescence one specimen was positive, while the other four specimens were negative.
4. 6 specimens gave discrepant results, 5 positive in IDEIA PCE Chlamydia and negative in IDEIA Chlamydia and one positive in IDEIA Chlamydia and negative in IDEIA PCE Chlamydia. On testing by direct immunofluorescence one of the 5 specimens positive in IDEIA PCE Chlamydia was positive, while the other four specimens were negative. The specimen positive in IDEIA Chlamydia and negative in IDEIA PCE Chlamydia was positive by direct immunofluorescence.

Appendix II: Correlation of IDEIA Chlamydia and IDEIA PCE Chlamydia with Cell Culture

The IDEIA PCE Chlamydia test has not been compared directly with cell culture. However, the increased prevalence rate observed with IDEIA PCE Chlamydia compared with IDEIA Chlamydia (Appendix I) can be used to calculate the predicted performance of IDEIA PCE Chlamydia versus cell culture.

Appendix II shows the known performance of IDEIA Chlamydia versus cell culture from external evaluations at four centres, and the predicted performance of IDEIA PCE Chlamydia versus cell culture based on its known performance versus IDEIA Chlamydia.

Specimen	Population	Performance of IDEIA Chlamydia[1] Versus Cell Culture		Predicted Performance of IDEIA PCE Chlamydia versus Cell Culture	
		Sensitivity %	Specificity %	Predicted Sensitivity (%)	Predicted Specificity (%)
Urogenital	High Risk	**91.9%** (385/419)	**98.5%** (1806/1834)	**100%**[2]	**98.5%**[3]
	Low Risk	**100%** (40/40)	**98.6%** (575/583)	**100%**[2]	**97.8%**[3]
Urine (Male)	High Risk	**84.7%** (149/176)	**98.7%** (938/950)	**93.38%**[2]	**97.8%**[3]

1. Based on external evaluations performed at four centres.
2. Predicted value calculated from increased positivity rate obtained with IDEIA PCE Chlamydia compared to IDEIA Chlamydia (Appendix I).
3. Predicted value calculated from specificity for IDEIA PCE Chlamydia data versus reference method (Appendix I).