EVALUATION OF THE OXOID DRYSPOT LEGIONELLA TEST RANGE

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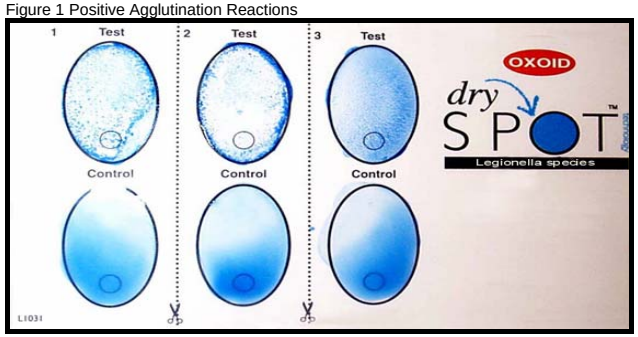
INTRODUCTION

Serological identification is the simplest way of identifying many species of *Legionella* for routine purposes. Latex agglutination kits utilising suspensions of latex beads coated in the appropriate antibodies have been available commercially for a number of years for this purpose. In the new Oxoid Dryspot Legionella kit test range, latex particles sensitised with specific *Legionella* antibody are dried onto a card. The *Legionella* strain under test is suspended directly from a fresh agar culture in a drop of a new low pH buffer and this is then used to resuspend the dried spot of sensitised latex beads. It is postulated that the buffer cleaves the lipid A component from LPS resulting in less stringy agglutination reactions than commonly occur when performing agglutinations using colonies taken directly from plates.

The test range consists of three latex agglutination kits, one for the identification of *L. pneumophila* serogroup 1(DR200M), a second for identification of *L. pneumophila* serogroups 2-14 (DR210M), and a third for identification of seven non-*pneumophila Legionella* species that have been implicated in human disease (DR220M). The seven *Legionella* species detected by this kit are *L. longbeachae* 1 & 2, *L. bozemanii* 1 & 2, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei, L. anisa*. Figure 1 below shows 3 positive agglutination reactions in the Legionella species kit (DR220M).

Figure 1 Positive Agglutination Reactions



METHODS

The kits were tested with pure cultures of *Legionella* (a mixture of clinical and environmental isolates) and other organisms. This was done in order to determine specificity and sensitivity against serological identity, which had been determined previously. The *Legionella* strains used in the evaluation were grown on buffered charcoal yeast extract agar. The non-*Legionella* strains were grown on blood agar.

The new tests were compared with a competitor's *Legionella* agglutination latex reagents. Comparison was not straightforward, however, as the competitor kits did not have reagents for non-*pneumophila Legionella,* also the competitor's reagents for *L pneumophila* serogroups 2-14 were individual reagents and not a single screening reagent. All kits were used according to their manufacturer's instructions.

RESULTS

A total of 201 strains was tested in order to determine the performance characteristics of the kits. Tables 1 – 3 show the sensitivities and tables 3 – 6, the specificities. Sensitivities and specificities were determined relative to serology. Table 7 shows the speeds of reaction of the kits in the evaluation.

Folio 766

Table 1 Sensitivities of the kits for detecting *L. pneumophila* Serogroup 1.

Kit	Strains tested	True positive results	% Sensitivity
Oxoid L. pneumophila sg1	50 x sg1	50	100
Competitor L. pneumophila sg1	50 x sg1	47	94

Table 2 Sensitivities of the kits for detecting *L. pneumophila* Serogroups 2-14

Kit	Strains tested	True positive results	% Sensitivity
Oxoid L. pneumophila sg2–14	51 x sg2 –14	51	100
Competitor L. pneumophila sg2–14	51 x sg2 –14	51*	100

* In order to compare the two kits, a positive result with any of the competitor sg 2-14 reagents was treated as a true positive, irrespective of whether the serological identification was correct.

Table 3 Sensitivities of the kits for detecting non-*pneumophila Legionella* species.

Kit	Strains tested	True positive results	% Sensitivity
Oxoid Legionella species	51 x NPL**	51	100

** NPL = non-*pneumophila Legionella* species

Table 4 Specificities of the kits for detecting *L. pneumophila* Serogroup 1.

Kit	Strains tested	False positive results	% Specificity
Oxoid L. pneumophila sg1	90 x (sg2–14, NPL, non Legionellae)	0	100
Competitor L. pneumophila sg1	90 x (sg2–14, NPL, non Legionellae)	0	100

Table 5 Specificities of the kits for detecting *L. pneumophila* Serogroups 2-14.

Kit	Strains tested	False positive results	% Specificity
Oxoid L.pneumophila sg2–14	89 x (sg1, NPL, non Legionellae)	2***	97.8
Competitor L. pneumophila sg2–14	89 x (sg1, NPL, non Legionellae)	21	76.4

***The two false positives with the Oxoid kit were one *L. pneumophila* Sg15 and one *L. pneumophila* Sg16 Data on these serogroups is currently limited, though it has been demonstrated that a common antigen is shared by serogroups 15 and 16 with *Legionella pneumophila* serogroups 3 and 4 respectively[1&2].

Table 6 Specificities of the kits for detecting non-*pneumophila Legionella* species.

Kit	Strains tested	False positive results	% Specificity
Oxoid Legionella species	90 x (sg1, NPL, non Legionellae.)	5****	94.4

****Four of the five false positives were Legionellae known to cross react[3] that are stated in the limitations section of the pack insert for the kit. The fifth false positive was a strain that was serologically identified as *L. micdadei* but grew on cysteine free-media.

Table 7 Speed of reaction for the various kits.

Time (s)	Kit				
	Oxoid L.p Sg1	Oxoid L.p Sg2-14	Oxoid species	Competitor L.p Sg1	Competitor L.p Sg2-14
0	86	66	76	35	18
10	100	98	92	67	56
20		100	98	84	75
30			100	93	92
40				100	98
50					100

DISCUSSION

The new Dryspot kits from Oxoid performed well in the evaluation and all gave satisfactory performance. Sensitivity for the three kits and specificity for the Sg1 kit were all 100%. The specificity of the Oxoid L. pneumophila Sg2-14 kit was less than 100 % because of reactions with *L. pneumophila* of serogroups15 and 16. This is not necessarily a disadvantage, but due to the rarity of serogroups 15 and 16 it is not yet clear whether this kit would be capable of detecting all such strains. The specificity of the Oxoid Dryspot Legionella species test kit was 94.4%. If reactions with the known cross reacting organisms[3] *L.parisiensis, L santicrucis, L tucsonensis* and *L. gratiana* are ignored, specificity improves to 98.8% for this kit.

The format of the Oxoid kits made them easy to use. In particular the large size of the oval reaction area together with the blue latex on a white background makes the Oxoid tests very easy to read. The "Dryspot" presentation was perceived as an advantage because the optimum quantity of test reagent was ready dispensed, unlike the competitor kit where the latex had to be dispensed. Often too much latex was accidentally dispensed with these kits, thus wasting expensive latex reagents.

There were far fewer reactions with the Oxoid kits than with the competitor kits where the result was difficult to determine because of "clumps" or "strings". This indicates that the new Oxoid buffer was effective at reducing stringiness.

Maximum reaction time for the Oxoid kits is 1 minute and for the competitor kits 2 minutes. Both manufacturers' kits, however, reacted well within these stated maximum times. Sixty five percent of the reactions with the Dryspot kits happened at time 0 *i.e.* on mixing. Over 90% of positive reaction with Oxoid kits had occurred within 10 seconds.

The Oxoid test range can be stored at room temperature for two years from the date of manufacture, this saves on refrigerator space and also means that the tests need not be warmed to room temperature before use.

In conclusion, the new Dryspot Legionella test range from Oxoid performed well in this evaluation and provides excellent alternatives to the other kits which are commercially available.

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Detection of *Legionella pneumophila* serogroup 15 by the Oxoid Legionella Latex Agglutination Kits

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INTRODUCTION

In response to queries from several customers concerning the ability of Oxoid's Legionella latex agglutination kits to detect *Legionella pneumophila* serogroups 15 and 16, it was decided to carry out a study to evaluate the performance of the Oxoid kits with available serotypes of *L. pneumophila* and strains of non *L. pneumophila Legionella* species.

Legionellaceae are Gram-negative, non-spore forming aerobic bacteria forming a family of at least 53[1] bacterial species that comprise in excess of 70 distinct serogroups. 20[2] of the 53 species have currently been implicated in causing a severe form of pneumonia called Legionnaires' Disease or legionellosis and an acute self-limiting disease termed Pontiac Fever.

Legionella spp are commonly found in aquatic environments such as cooling towers, air conditioning appliances, and domestic plumbing systems and to a lesser extent soil.

The main pathogenic species of the genus *L. pneumophila* has been shown to contain three distinct sub species based on DNA hybridisation and biochemical studies.[3] These are *L. pneumophila* subsp *pneumophila*, *L. pneumophila* subsp *fraseri* and *L. pneumophila* subsp *pascullei*. Immunologically, 15 individual serogroups are currently recognised in this classification, of which serogroup 1 causes around 80-90% of clinical cases of Legionnaires' disease.

L. pneumophila serogroups 2 to 15 and the non *pneumophila* species of *Legionella* have only rarely been isolated. The majority of infections caused by these strains are (in decreasing incidence) from serogroups 6, 3, 10, 4 and 5. *L. pneumophila* serogroup 15 has not currently been isolated from clinical or environmental samples in Europe[4] and has only been isolated once in the USA.[3] This most recent serogroup to be designated contains only one strain (Lansing-3 (ATCC® 35251)). A 16th serogroup was proposed from studies of the Jena-1 isolate[5,6] until further analysis showed that the strain did not form a unique serogroup but was a member of serogroup 4 *L. pneumophila* (monoclonal group Portland 1).[7,8]

The Oxoid Legionella latex agglutination kits provide a rapid means of identifying cultures as pathogenic *Legionella* from both environmental and clinical samples. The Oxoid kits include reagents to specifically detect *L. pneumophila* Serogroups 1 and 2-14/15. A further reagent allows the identification of seven pathogenic non-*L. pneumophila* species.

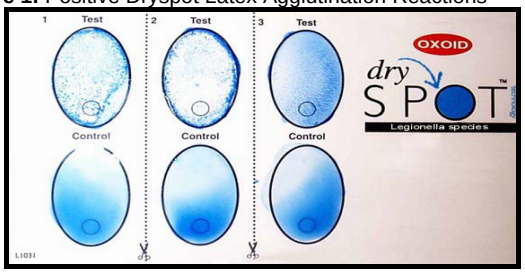
MATERIALS AND METHODS

16 *L. pneumophila* serogroup 1, 25 *L. pneumophila* serogroup 2-14, 55 non-*L. pneumophila* (including 24 non-*L. pneumophila Legionella* species strains capable of being detected with the Oxoid species latex reagent) and a serogroup 15 isolate (Lansing-3) ATCC® 35251 were analysed with the Oxoid latex agglutination kit (DR0800M) and the Dryspot™ latex agglutination kits (DR0200M, DR0210M and DR0220M).

All strains originated from national culture collections and were grown as pure cultures on Oxoid BCYE Medium (PO5072A) for 48 hours at 37°C.

All cultures were tested with the Oxoid latex agglutination kits according to the manufacturer's instructions.

Figure 1: Positive Dryspot Latex Agglutination Reactions



RESULTS AND DISCUSSION

The results show that the antibodies used in the Oxoid *L. pneumophila* 2-14 latex reagent of both kits (DR0800M and DR0210M) also detects the Lansing 3 isolate of *L. pneumophila*. The Lansing 3 strain has been shown to be genetically closely related to some serogroup 4 strains of *L. pneumophila.*[3]

Table 1: Results for the Oxoid Legionella Latex Test Kit (DR0800M).

	Pneumophila 1 reagent	Pneumophila 2-14 reagent	NLP Reagent
Sensitivity	**100%** (16/16)	**100% *** (26/26)	**87.5%** (21/24)
Specificity	**97.5%** (79/81)	**98.6%** (70/71)	**94.5%** (69/73)

Table 2: Results for the Oxoid Dryspot Legionella Latex Test (DR0200M, DR0210M and DR0220M).

	Pneumophila 1 reagent	Pneumophila 2-14 reagent	NLP Reagent
Sensitivity	**100%** (16/16)	**100%*** (26/26)	**87.5%** (21/24)
Specificity	**96.3%** (78/81)	**94.4%** (67/71)	**94.3%** (69/73)

NLP Reagent=Non *L. pneumophila* species Reagent
*****Including the *L. pneumophila* 15 strain.

The *L. pneumophila* 1 and 2-14 reagents of both latex kits demonstrated excellent sensitivity and specificity (tables 1 and 2) with the strains of serogroups 1-14 and the *Legionella* species strains included in the test panel. Results obtained with the Species Test reagent were affected by known and reported cross reactions with other serogroups of non-*Legionella pneumophila* organisms.[9]

CONCLUSIONS

The Oxoid latex agglutination kits are accurate tests that are able to detect the *Legionella pneumophila* serogroup 15 (ATCC® 35251) as well as detecting *L. pneumophila* 1-14 and other pathogenic non-*L. pneumophila*, *Legionella* strains. However, as only one serogroup 15 strain is available for testing, it is not thought appropriate to rename the serogroup 2-14 latex reagent based on the results from a single isolate.

The Oxoid kits benefit the user by allowing discrimination of samples into three groups: *L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2-15 (with the 2-14 reagent) and other *Legionella* species in a fast and simple screening procedure.

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